US-PAT-NO: 4832916
DOCUMENT-IDENTIFIER: US 4832916 A
TITLE: Chromatographic column for immunological determining methods
DATE-ISSUED: May 23, 1989
US-CL-CURRENT: 422/70,210/656 ,210/658 ,436/541 ,436/807 ,436/810
APPL-NO: 7/ 018085
DATE FILED: February 24, 1987
FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: DE 3608883
FOREIGN-PRIORITY-APPL-DATE: March 17, 1986
DEPR:
Whereas in the design of FIG. 1 the sleeve widening of the long filter is substantially in metal over a relatively short section, for the luminescence method only a short piece of filter 56, possibly with plastic outer sheath, is held in for example a long metallic sheath tube 57. A widened sleeve 59 consisting for example of plastic is attached in a similar manner to FIG. 1 over the complementary funnel-shaped bottom 58 of the column. However, in this case the widened sheath/shield 59 extends right to the top at the intermediate or annular space formed between the inner side of the straight column tube and the outer side of the funnel-shaped bottom 58 and is contained snuggly fitted in the latter on slight application pressure. The possibly metallic sheath tube 57 consisting of galvanized copper for locating the filter serves as shield. In the illustration the column is cut inclined at the bottom to ensure in constrained manner a dripping of the liquid possibly present only in small quantities. Embodiments without a shield 59 are also possible. The filter paper can also be pressed into the capillary sheath. For fluorescence-immunological investigation the reagents are pipette into the reaction portion 60 of the chromatographic column, including the marking substance. After setting the reaction equilibrium the funnel-shaped bottom of the reaction portion is perforated up to the filter paper lying closely

US-PAT-NO: 5316732
DOCUMENT-IDENTIFIER: US 5316732 A
TITLE: Extraction vial
DATE-ISSUED: May 31, 1994
US-CL-CURRENT: 422/102,206/438 ,206/570 ,422/101 ,422/99
,435/287.1 ,435/288.1
,435/308.1 ,D24/224 ,D9/443
APPL-NO: 7/ 907359
DATE FILED: July 1, 1992
BSPR:
An alternative to this approach is to utilize extraction or pretreatment
reactions whereby the sample material is added to a liquid extracting medium
such that the sample is brought from its solid or semi-solid form to a liquid
form. Conventionally, this is accomplished by a technician in a laboratory
setting and is carried out in small transfer vessels, whereby the extraction
medium containing the sample is added to the chromatographic medium via, e.g.,
a pipette. This scenario raises additional problems, particularly in the areas
of contamination and waste disposal. Such problems are particularly relevant
with respect to the possibility of transfer of communicable diseases to the
technician(s), as well as the potential for cross-contamination of samples.

US-PAT-NO: 4863610
DOCUMENT-IDENTIFIER: US 4863610 A
TITLE: Scraping, collecting & eluting apparatus for thin layer chromatography
and method for its use
DATE-ISSUED: September 5, 1989
US-CL-CURRENT: 210/658,210/198.3 ,210/772 ,422/101 ,422/61 ,422/70 ,436/178
APPL-NO: 7/ 060402
DATE FILED: June 11, 1987
BSPR:
Glass pasteur pipettes were plugged with cotton or glass wool to allow air flow
but not adsorbent flow through the pipette. The tapered end of the pipette was
used to scrape the adsorbent from the thin layer chromatography plate while the
other end of the pipette was attached to a vacuum tubing. The scraped
adsorbent was aspirated into the pipette and embedded in the glass wool or
cotton. The loss of adsorbent during scraping and collection was lessened.
This decreased the drudgery of the scraping process although the lack of
flexibility in the metal contact with the plate mentioned earlier is of equal
if not greater detriment in the glass to plate contact. Additionally, the tip
of the pipette would easily break if too much pressure was applied to the thin
layer chromatography plate with the pipette, and the small diameter of the tip
opening made scraping slow and tedious because the volume of adsorbent removed
with each pass was very small.

therebelow and the liquid reaction mixture flows from above into the filter column. When this is done the components are separated in that the free phase is bonded i the upper column portion and the bonded phase, i.e. the antigen/antibody/complex to be measured, passes through the separating column and drips down onto the bottom of the test tube where it can thus be measured by extinction photometry. The shield 59 can be coloured. FIG. 3 shows an embodiment comprising a measuring cuvette 66 which is formed as microcuvette and in order to be usable for very small amounts of filtered liquid has a constriction in the lower region 68. Of interest is that the same column 70 with practically the same funnel-shaped bottom 72 as described with regard to FIGS. 1 and 2 fits into said measuring cuvette and is simply inserted with its lower open end 74. In this case the filter material 76 is pressed into a plastic sheath 78 which bears closely on the perforable bottom 80 of the funnel-shaped reaction vessel. The reaction vessel can once again be sealed by a cover 40. As in FIG. 1 the lower end of the sheath tube, in this case the microcuvette forms the measuring vessel and the column 70 the reaction and separating vessel. The antigen/antibody/complex can again be measured photometrically. In this case no separate shield is provided but this can be done. The microcuvette is a design with particularly small bottom 82 for measuring technical reasons.

US-PAT-NO: 4526686
DOCUMENT-IDENTIFIER: US 4526686 A
TITLE: Apparatus for chromatographic sample injection
DATE-ISSUED: July 2, 1985
US-CL-CURRENT: 210/198.2,210/198.3 ,96/105
DISCLAIMER DATE: 20000920
APPL-NO: 6/ 533899
DATE FILED: September 20, 1983
PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 304,780, filed Sept. 23, 1981, now U.S. Pat. No. 4,405,344 issued Sept. 20, 1983, the disclosure of which is incorporated herein by reference in its entirety.

FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: IT 25018 A/80
FOREIGN-PRIORITY-APPL-DATE: September 30, 1980
BSPR:
The present invention relates to an apparatus to perform sampling in chromatographic systems with very small amounts of liquid sample, said apparatus being particularly applicable to high resolution gas chromatographic systems with cold injection, using capillary or micropacked columns, to high resolution liquid chromatographic systems, or to thin-layer chromatographic systems. Use of this apparatus makes it possible to perform controllable and reproducible sampling on very small amounts of sample, with values unattainable through the techniques usually employed for liquid sampling in chromatographic systems and particularly using micro-syringes or pipettes.

US-PAT-NO: 4405344
DOCUMENT-IDENTIFIER: US 4405344 A
TITLE: Method and equipment for volumetrically controlled and reproducible introduction of small amounts of liquid samples into chromatographic analysis systems
DATE-ISSUED: September 20, 1983
US-CL-CURRENT: 95/89,96/105
APPL-NO: 6/ 304780
DATE FILED: September 23, 1981
FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: IT 25018 A/80
FOREIGN-PRIORITY-APPL-DATE: September 30, 1980
BSPR:
The present invention relates to a method and an apparatus to perform sampling in chromatographic systems with very small amounts of liquid sample, said method and said equipment being particularly but not exclusively applicable to high resolution gas chromatographic systems, with capillary or micropacked columns, or to thin-layer chromatographic systems. Use of this method and apparatus makes it possible to perform controllable and reproducible sampling of very small amounts of sample, with values unattainable through the techniques usually employed for liquid sampling in chromatographic systems and particularly using micro-syringes or pipettes.

US-PAT-NO: 6190559
DOCUMENT-IDENTIFIER: US 6190559 B1
TITLE: Evaporative packing a capillary columns
DATE-ISSUED: February 20, 2001
US-CL-CURRENT: 210/656,210/198.2 ,210/510.1 ,95/82 ,95/88 ,96/101
APPL-NO: 9/ 365610
DATE FILED: August 2, 1999
PARENT-CASE:
This application is a division of Ser. No. 09/087;202, filed May 29, 1998, now
U.S. Pat. No. 5,997,746.

DEPR:
Alternatively, the slurry can be transferred from the vessel in which it was
prepared into another vessel, such as a vessel having a conical bottom with a
hole at the apex of the conical bottom, such as is shown in FIG. 8. One such
vessel which can be used in accordance with the invention, is a common
polyethylene pipette tip. In yet another embodiment of the invention, a vessel
having a conical tip with a hole in the apex of the cone and a septum in the
hole can be used. When using such vessels, the capillary tube is inserted into
the slurry through the hole or, if the hole in the vessel is equipped with a
septum, through the septum.

DEPR:
A slurry of 5 .mu.m diameter solid glass microspheres was prepared by mixing
0.1 gram of the spheres with 0.5 milliliters of 100% methanol in a small (1 ml)
glass vial. The mixture was stirred thoroughly for 5 minutes, ultrasonicated
for 5 minutes, and then allowed to settle for 2 hours. After removing the
excess solvent above the slurry, which had settled to the bottom of the vial,
approximately 10 .mu.l of slurry was transferred to a polyethylene vessel (a
commercial polyethylene pipette tip) possessing a conical bottom having a 0.5
mm hole at it's apex. The slurry was again allowed to settle for approximately
5 minutes, while holding the pipette tip in the vertical

position.

DEPR:
Both the slurry-containing vessel and the silica needle were mounted horizontally on the stage of a standard light microscope and viewed at 100-.times. magnification. The microscope stage had an additional translation stage that allowed the silica needle to be moved into the hole of the pipette tip. By moving the tip into brief contact (.apprxeq.0.5 seconds), with the slurry through the hole, approximately 75-100 microspheres were transferred into the silica needle.

DEPR:
A slurry was prepared as in Example 1. After settling, 100 .mu.l of slurry was transferred to a polyethylene pipette tip. The tip was held in the vertical position and the slurry allowed to settle for 15 minutes. Settling time was monitored with a light microscope to ensure that >95% of the material had settled out to the bottom of the tip.

DEPR:
While left in the vertical position, the silica capillary tube was inserted approximately 50 .mu.m into the hole at the bottom of the pipette tip. The dense (nearly opaque) slurry filled the silica tube by capillary action. The tube was left in contact with the slurry until 15 cm of the tube was filled.

CCXR:
210/198.2

US-PAT-NO: 4526686
DOCUMENT-IDENTIFIER: US 4526686 A
TITLE: Apparatus for chromatographic sample injection
DATE-ISSUED: July 2, 1985
US-CL-CURRENT: 210/198.2,210/198.3 ,96/105
DISCLAIMER DATE: 20000920
APPL-NO: 6/ 533899
DATE FILED: September 20, 1983
PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 304,780, filed Sept. 23, 1981, now U.S. Pat. No. 4,405,344 issued Sept. 20, 1983, the disclosure of which is incorporated herein by reference in its entirety.

FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY-APPL-NO: IT 25018 A/80
FOREIGN-PRIORITY-APPL-DATE: September 30, 1980

ABPL:
An apparatus for controllably and reproducibly introducing, small amounts of a liquid sample into chromatographic systems, especially high resolution gas chromatographic systems with cold injection, thin-layer chromatographic systems and high resolution liquid chromatographic systems, in order to obtain sampling extremely reduced in volume and presenting maximum reliability and reproducibility, uses a sample container having a pipette-like or nozzle-like outlet neck of very small diameter. The liquid placed in the container is submitted to at least one pressure pulse which is controlled in duration and/or amplitude in order to determine emission of a corresponding and controlled quantity of liquid from the outlet neck.

BSPR:
The present invention relates to an apparatus to perform sampling in chromatographic systems with very small amounts of liquid sample, said apparatus being particularly applicable to high resolution gas chromatographic systems with cold injection, using capillary or micropacked columns, to high

resolution liquid chromatographic systems, or to thin-layer chromatographic systems. Use of this apparatus makes it possible to perform controllable and reproducible sampling on very small amounts of sample, with values unattainable through the techniques usually employed for liquid sampling in chromatographic systems and particularly using micro-syringes or pipettes.

BSPR:
Both these injection systems use syringes, microsyringes or pipettes as will be described later on.

BSPR:
The microsyringes used in chromatography are generally of the type with calibrated body (capable of sampling amounts ranging from 0.2 to 10 microliters) or of the type with calibrated needle, where the piston penetrates into the needle. The latter microsyringes are capable of handling smaller quantities of samples, in a reliable and reproducible way, but only within certain limits, in particular with lower limits of about 100-200 nanoliters. Below this limit, the high surface tension of the liquid and the relatively reduced speed of the piston movement do not allow the drop, which has formed at the needle end, to fall from it, considering the reduced diameter of the outlet nozzle of the needle. Precision is moreover negatively affected by poor sealing between piston and calibrated needle. Another known system is the sampling system commonly used in the laboratory and named "pipette system", in which a calibrated tubing is filled with a liquid to be transferred by filling the pipette due to capillary forces or by sucking it into the tubing. The liquid amount placed in the tubing is retained in the tubing by the capillary forces or by closing one end when liquid aspiration has been carried out. Then, an injection of the liquid is made by opening said end or pushing the liquid by the carrier gas. This sampling method or transfer

method of
determined amounts of liquid is well known and has been used in gas
chromatography too, but however only for quantities usually measurable in a
rather rough way. The literature reports a lower limit of 25-50 nanoliters
(see R. Kaiser--Gas Phase Chromatography--Vol. I pp. 90-95--Butterworths
1963--London) but thise limits are difficult to reach and anyhow require small
tubes filled exclusively due to capillary forces. This implies that the volume
of liquid injection is difficult to control and reproduce.

BSPR:
It will be seen from the above comparison that the prior art sample injection
devices operate by controlling sample size in the filling step and injecting
the entire contents of the filled injection system using pressure in a
different way from the way in which it is employed in the present invention.
Even a combination of the cited references would only lead the skilled art
worker to a pipette of the type shown in FIG. 26 or 27 of Kaiser which,
however, is actuated by a pulsed valve. This type of device is typical of a
titration burette which commonly delivers minimum sample quantities of 0.1-0.2
milliliters, and is unable to deliver samples in the picoliter or nanoliter
volume range.

BSPR:
a sample container having a volume grater than a desired liquid sample volume
and having a pipette--or nozzle-shaped neck at its outlet, terminating in an
opening with a diameter of 1-100 .mu.m;

DEPR:
The apparatus of the invention is based on the use of a container for the
liquid sample which has a volume greater than that of the sample to be
injected, said container being made of any suitable material, for instance
glass, metal, fused silica or any other material, and having a

pipette shape, a
syringe needle shape or any other suitable shape.

DEPR:
The essential condition is that said container, as indicated by 10 or 12 in
FIG. 1, presents a neck defining an outlet having a maximum size (a diameter d
in this specific case) ranging from 1 to 100 .mu.m, preferably between 1 and 30
.mu.m. Said neck can be nozzle-shaped, as indicated by 14 in FIG. 1, and
therefore has a preferred neck diameter d from 10 to 30 .mu.m, or micropipette-shaped, as indicated by 16 in the same FIG. 1, with a preferred
diameter d from 1 to 20 .mu.m. The container, 10 or 12 as illustrated in FIG.
1, can be filled by the usual methods, for instance by means of a syringe, by
gravity, or in any way whatsoever, with a quantity of liquid 18 which is
greater than the volume that is required to flow from the neck 14 or 16 to be
analyzed in the chromatographic system. Once the container 10 or 12 has been
fed with the sample 18, it is necessary to check, especially in the case of the
container 12 with a pipette-like end, that the liquid goes as far as to reach
the neck 14 or 16, forming a meniscus therein. Under these conditions, taking
into account the reduced size of the neck, the surface tension of the liquid
prevents the latter from flowing out of the neck, forming drops, this obviously
provided that the neck 14 or 16 does not touch foreign bodies, which may help
the liquid to flow outside the container.

CLPV:
a sample container having a volume greater than a desired liquid sample volume
and having a pipette- or nozzle-shaped neck at its outlet, terminating in an
opening with a diameter of 1-100 .mu.m;

CCOR:
210/198.2

	Type	L #	Hits	Search Text	DBs	Time Stamp
1	BRS	L1	9	pipette near5 perforation	USPAT	2002/01/02 14:17
2	BRS	L2	44	pipette near5 chromatogra$4	USPAT	2002/01/02 14:34
3	BRS	L3	12121	pipette	USPAT	2002/01/02 14:34
4	BRS	L4	1553	210/198.2.ccls.	USPAT	2002/01/02 14:34
5	BRS	L5	47	3 and 4	USPAT	2002/01/02 14:51
6	BRS	L6	67	fritless	USPAT	2002/01/02 14:52
7	BRS	L7	2	4 and 6	USPAT	2002/01/02 14:54
8	BRS	L8	0	3 and 6	USPAT	2002/01/02 14:54
9	BRS	L9	21095	incision	USPAT	2002/01/02 14:54
10	BRS	L10	0	4 and 9	USPAT	2002/01/02 14:55
11	BRS	L11	3	pipette near5 incision	USPAT	2002/01/02 14:56
12	BRS	L12	0	perforat4$ near5 column	USPAT	2002/01/02 14:57
13	BRS	L13	1571	perforat$4 near5 column	USPAT	2002/01/02 14:59
14	BRS	L14	176623	chromatogra$4	USPAT	2002/01/02 14:59
15	BRS	L15	278	13 and 14	USPAT	2002/01/02 14:59
16	BRS	L16	114	(perforat$4 near5 column) same chromatogra$4	USPAT	2002/01/02 15:01
17	BRS	L17	94	perforat$4 adj column	USPAT	2002/01/02 15:02
18	BRS	L18	8	14 and 17	USPAT	2002/01/02 15:02

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1	9	pipette near5 perforation	USPAT	2002/01/02 14:17
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3	12121	pipette	USPAT	2002/01/02 14:34
4	1553	210/198.2.ccls.	USPAT	2002/01/02 14:34
5	47	pipette and 210/198.2.ccls.	USPAT	2002/01/02 14:51
6	67	fritless	USPAT	2002/01/02 14:52
7	2	210/198.2.ccls. and fritless	USPAT	2002/01/02 14:54
8	0	pipette and fritless	USPAT	2002/01/02 14:54
9	21095	incision	USPAT	2002/01/02 14:54
10	0	210/198.2.ccls. and incision	USPAT	2002/01/02 14:55
11	3	pipette near5 incision	USPAT	2002/01/02 14:56
12	0	perforat4$ near5 column	USPAT	2002/01/02 14:57
13	1571	perforat$4 near5 column	USPAT	2002/01/02 14:59
14	176623	chromatogra$4	USPAT	2002/01/02 14:59
15	278	(perforat$4 near5 column) and chromatogra$4	USPAT	2002/01/02 14:59
16	114	(perforat$4 near5 column) same chromatogra$4	USPAT	2002/01/02 15:01
17	94	perforat$4 adj column	USPAT	2002/01/02 15:02
18	8	chromatogra$4 and (perforat$4 adj column)	USPAT	2002/01/02 15:02

	Type	L #	Hits	Search Text	DBs	Time Stamp
1	BRS	L1	9	pipette near5 perforation	USPAT	2002/01/02 14:17
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3	BRS	L3	12121	pipette	USPAT	2002/01/02 14:34
4	BRS	L4	1553	210/198.2.ccls.	USPAT	2002/01/02 14:34
5	BRS	L5	47	3 and 4	USPAT	2002/01/02 14:51
6	BRS	L6	67	fritless	USPAT	2002/01/02 14:52
7	BRS	L7	2	4 and 6	USPAT	2002/01/02 14:54
8	BRS	L8	0	3 and 6	USPAT	2002/01/02 14:54
9	BRS	L9	21095	incision	USPAT	2002/01/02 14:54
10	BRS	L10	0	4 and 9	USPAT	2002/01/02 14:55
11	BRS	L11	3	pipette near5 incision	USPAT	2002/01/02 14:56
12	BRS	L12	0	perforat4$ near5 column	USPAT	2002/01/02 14:57
13	BRS	L13	1571	perforat$4 near5 column	USPAT	2002/01/02 14:59
14	BRS	L14	176623	chromatogra$4	USPAT	2002/01/02 14:59
15	BRS	L15	278	13 and 14	USPAT	2002/01/02 14:59
16	BRS	L16	114	(perforat$4 near5 column) same chromatogra$4	USPAT	2002/01/02 15:01
17	BRS	L17	94	perforat$4 adj column	USPAT	2002/01/02 15:02
18	BRS	L18	8	14 and 17	USPAT	2002/01/02 15:02
19	BRS	L19	26	septumless	USPAT	2002/01/02 15:11
20	BRS	L20	0	4 and 19	USPAT	2002/01/02 15:11
21	BRS	L21	79702	plunger	USPAT	2002/01/02 15:13
22	BRS	L22	144	4 and 21	USPAT	2002/01/02 15:13

	Comments	Error Definition	Errors
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L Number	Hits	Search Text	DB	Time stamp
1	9	pipette near5 perforation	USPAT	2002/01/02 14:17
2	44	pipette near5 chromatogra$4	USPAT	2002/01/02 14:34
3	12121	pipette	USPAT	2002/01/02 14:34
4	1553	210/198.2.ccls.	USPAT	2002/01/02 14:34
5	47	pipette and 210/198.2.ccls.	USPAT	2002/01/02 14:51
6	67	fritless	USPAT	2002/01/02 14:52
7	2	210/198.2.ccls. and fritless	USPAT	2002/01/02 14:54
8	0	pipette and fritless	USPAT	2002/01/02 14:54
9	21095	incision	USPAT	2002/01/02 14:54
10	0	210/198.2.ccls. and incision	USPAT	2002/01/02 14:55
11	3	pipette near5 incision	USPAT	2002/01/02 14:56
12	0	perforat4$ near5 column	USPAT	2002/01/02 14:57
13	1571	perforat$4 near5 column	USPAT	2002/01/02 14:59
14	176623	chromatogra$4	USPAT	2002/01/02 14:59
15	278	(perforat$4 near5 column) and chromatogra$4	USPAT	2002/01/02 14:59
16	114	(perforat$4 near5 column) same chromatogra$4	USPAT	2002/01/02 15:01
17	94	perforat$4 adj column	USPAT	2002/01/02 15:02
18	8	chromatogra$4 and (perforat$4 adj column)	USPAT	2002/01/02 15:02
19	26	septumless	USPAT	2002/01/02 15:11
20	0	210/198.2.ccls. and septumless	USPAT	2002/01/02 15:11
21	79702	plunger	USPAT	2002/01/02 15:13
22	144	210/198.2.ccls. and plunger	USPAT	2002/01/02 15:13

US005997746A

United States Patent [19]

Valaskovic

[11] Patent Number: 5,997,746

[45] Date of Patent: Dec. 7, 1999

[54] **EVAPORATIVE PACKING OF CAPILLARY COLUMNS**

[75] Inventor: **Gary A. Valaskovic**, Cambridge, Mass.

[73] Assignee: **New Objective Inc.**, Cambridge, Mass.

[21] Appl. No.: **09/087,202**

[22] Filed: **May 29, 1998**

[51] **Int. Cl.**[6] **B01D 15/08**

[52] **U.S. Cl.** **210/656**; 210/198.2; 210/510.1; 95/82; 95/88; 96/101

[58] **Field of Search** 210/635, 656, 210/658, 198.2, 510.1; 95/88, 82; 96/101; 141/12, 80

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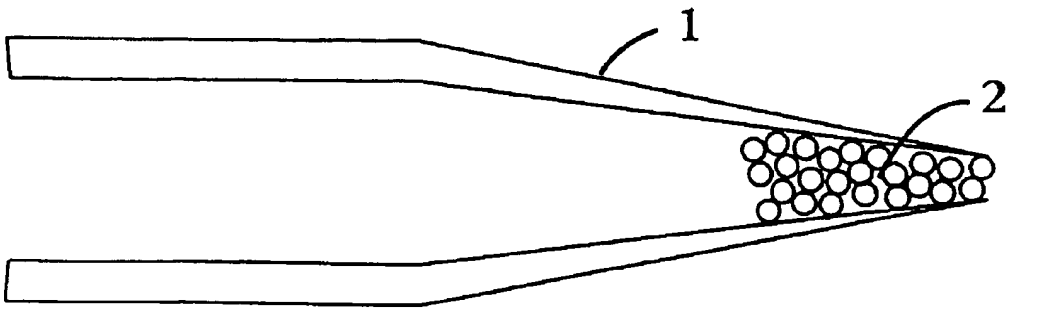
Primary Examiner—Ernest G. Therkorn

Attorney, Agent, or Firm—Sprung Kramer Schaefer & Briscoe

[57] **ABSTRACT**

Method for loading a column with a packing material by inserting one end of a column to be packed into a slurry of a packing material in a volatile solvent, allowing said slurry to be drawn into said end of said column by capillary action, withdrawing said end from said slurry, and removing said volatile solvent from the slurry that has been drawn into said end of said column, through the same end of the column at which the slurry entered, and sinteriing.

5 Claims, 5 Drawing Sheets



READY FOR SINTERING

Fig. 1

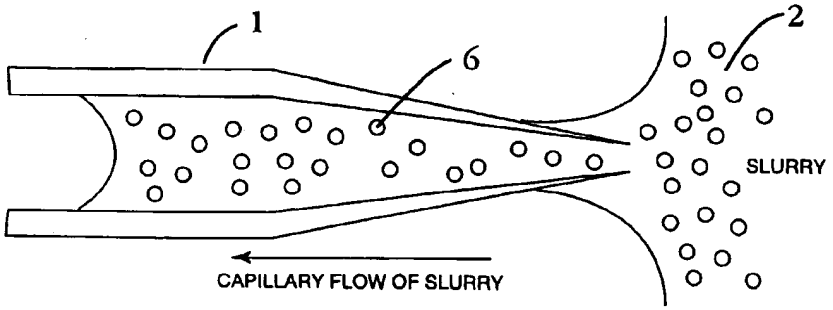


Fig. 2

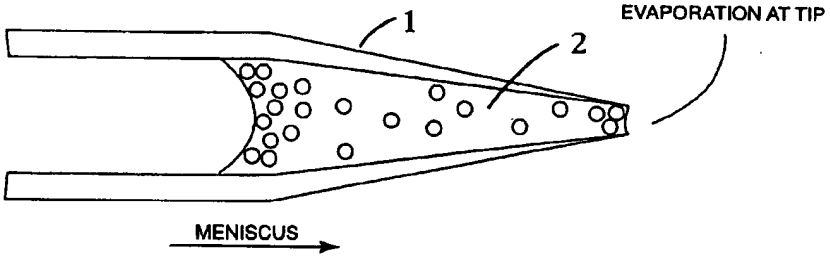


Fig. 3

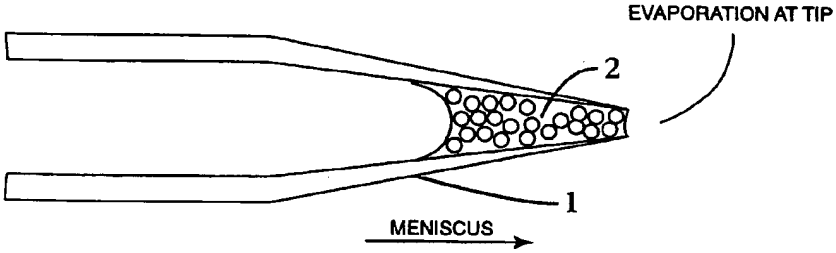
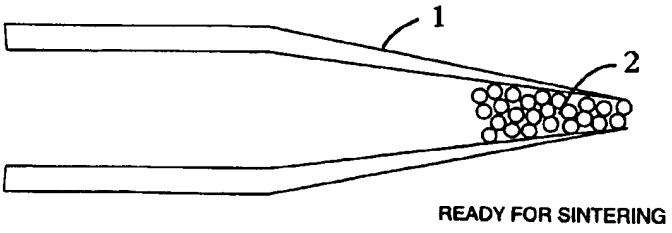


Fig. 4



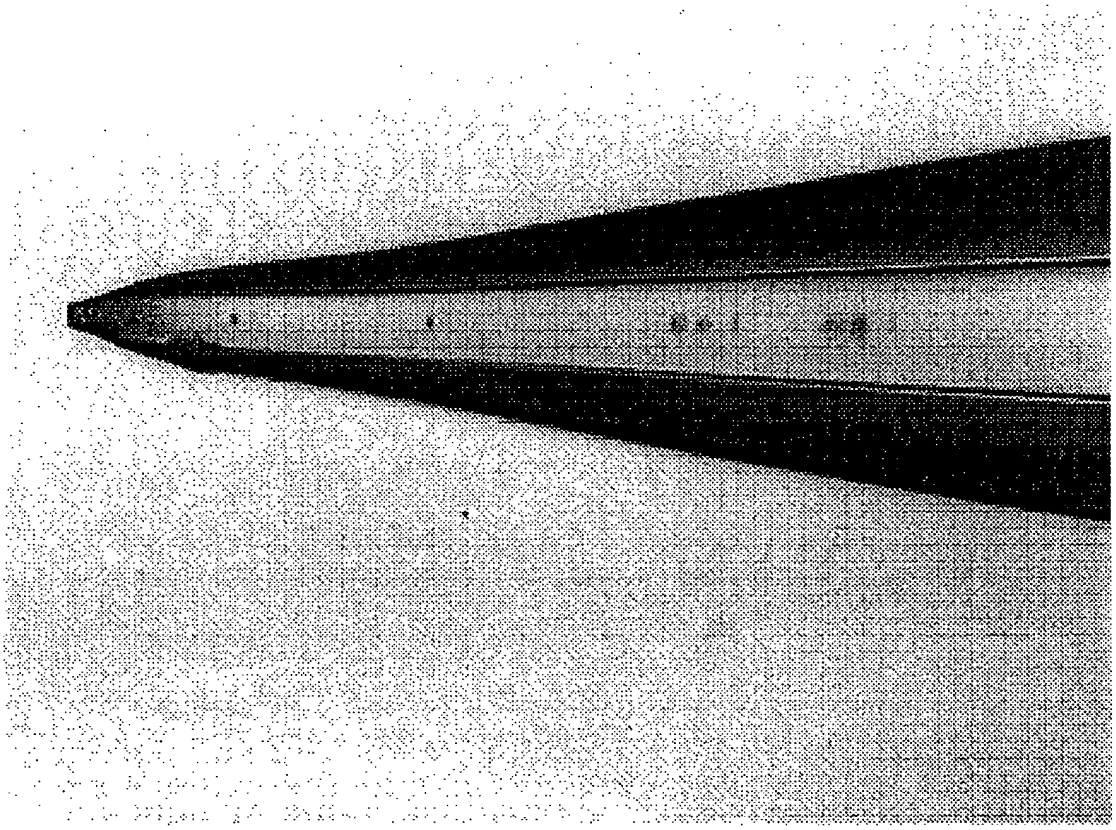


Fig. 5

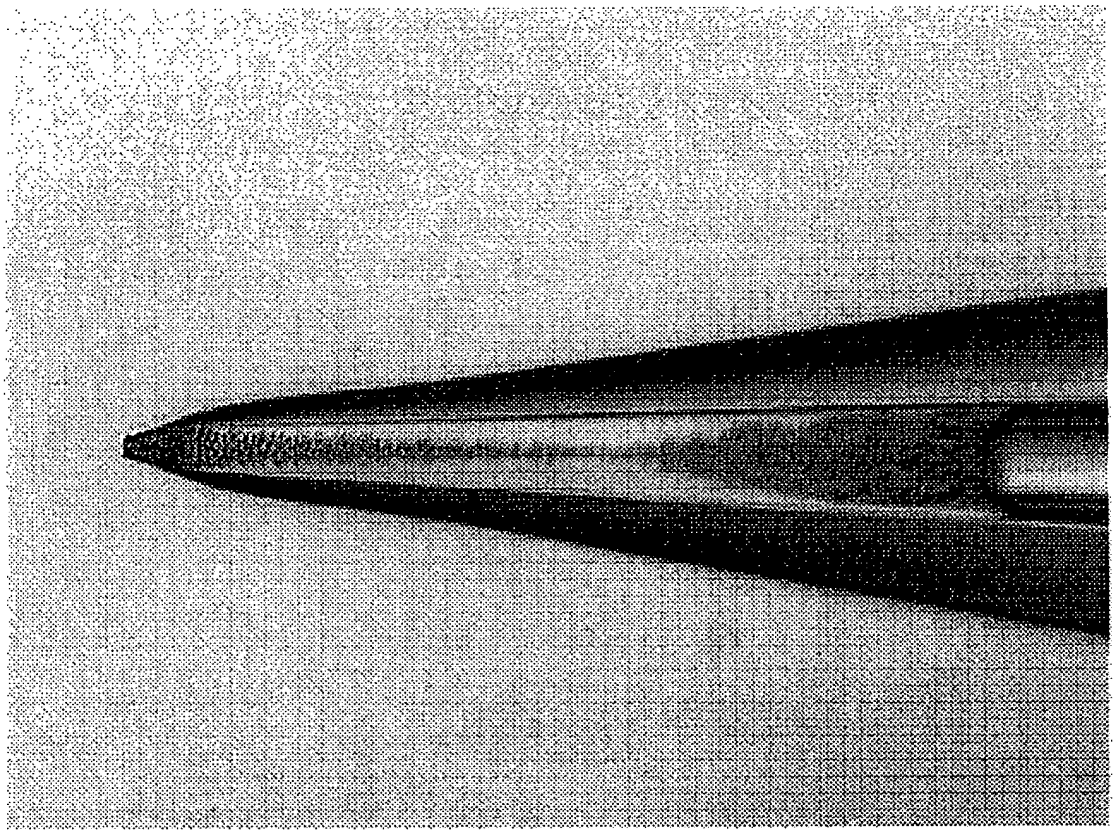


Fig. 6

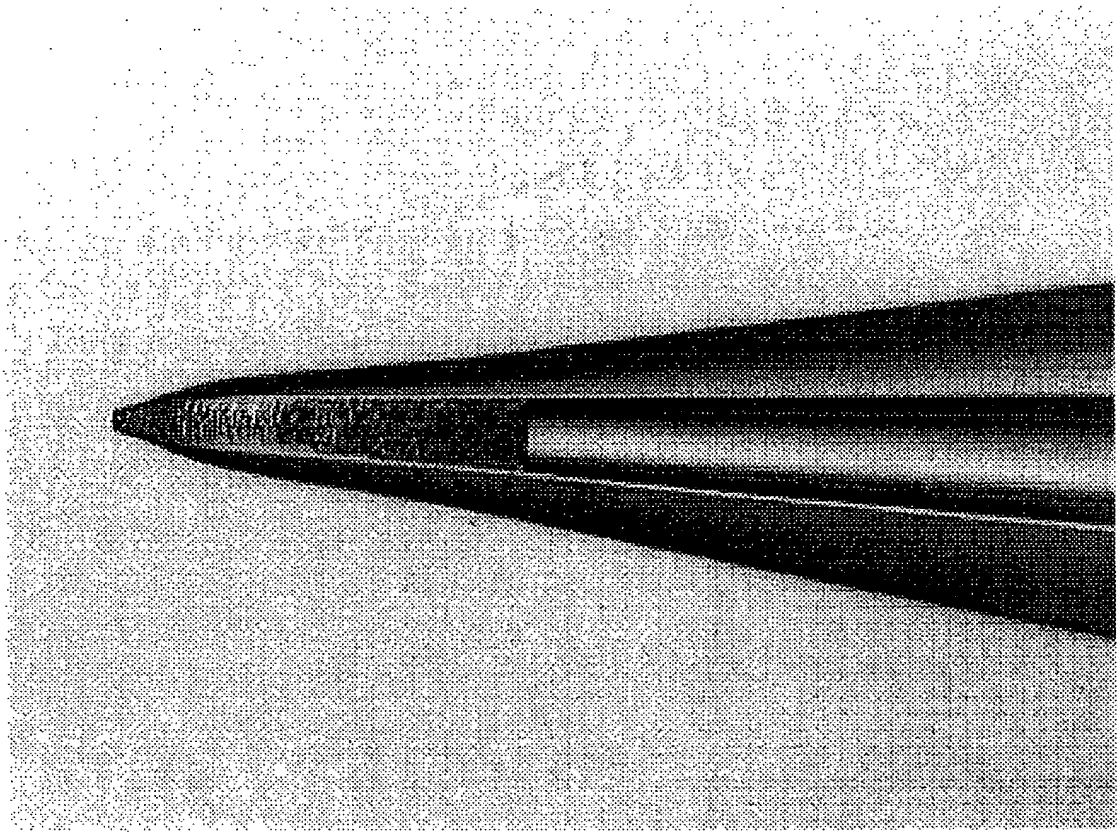
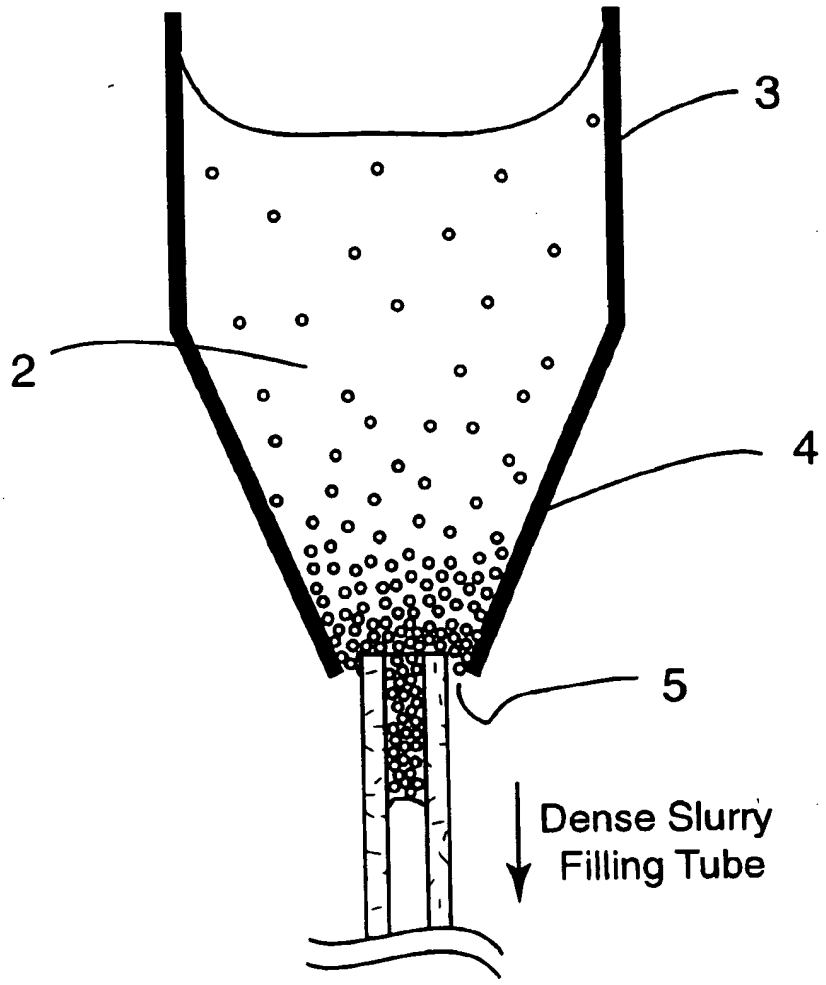


Fig. 7



Filling a tube with dense slurry by capillary action

Fig. 8

EVAPORATIVE PACKING OF CAPILLARY COLUMNS

BACKGROUND OF THE INVENTION

This invention pertains to a novel method of packing capillary columns. More particularly, the invention pertains to a method of packing capillary columns by drawing a slurry of packing material into a capillary column through capillary action, and then removing solvent from the slurry through one end of the column.

There are a variety of methods currently in use for packing capillary columns, such as those columns used in the fields of chromatography and electrospray ionization mass spectrometry (ESI-MS). The most popular methods in current use are the so-called "slurry packing" methods.

U.S. Pat. No. 5,679,255 discloses a method whereby a retaining material, such as a ceramic frit which will allow solvent, but not packing material, to pass is placed in one end of the column. A slurry of polymeric packing material in an organic solvent, such as THF, is then pumped through the column, from the end opposite that having the retaining frit. The packing material thereby accumulates in the column, while the liquid portion of the slurry passes out through the frit. This method has certain disadvantages, however. The capillary tubing used must be capable of withstanding the pressure generated by the pumping of the slurry into the tube, and necessary equipment, such as a pump and solvent recovery system must be provided. High packing pressure may also cause deterioration of or damage to the packing material.

U.S. Pat. No. 4,483,773 discloses a method wherein an end restriction is first placed in a column, to permit the flow of solvent, but restrict the passage of particles out of the end of the column. A slurry is then caused to flow into the column, under pressure. A two-step pressure sequence is then used to first fill up the column and form a bed of particles and then to uniformly compress the bed. This method is less than completely satisfactory, because special equipment is required to practice it.

There is therefore a need for a simple, direct method for packing capillary columns, which does not rely on special equipment for pumping or pressurizing slurries into the columns.

SUMMARY OF THE INVENTION

It has now been discovered that a slurry of packing material can be drawn up into a capillary column through capillary action and that when the solvent in the slurry is evaporated out of the slurry through the same end of the column through which the slurry entered, the packing that is originally suspended in the slurry migrates towards the end of the column to become closely packed.

In accordance with the invention, there is therefore provided a method for packing a capillary column which comprises forming a slurry of the packing material to be packed into the column, placing the slurry in a vessel, inserting one end of a capillary column into said slurry in said vessel or reservoir, drawing slurry into said column by capillary action and removing the solvent from the slurry that has been drawn into the column through the same end through which it entered the column. Preferably, the solvent is removed only from the end through which it entered the column, and it is particularly preferable that the solvent be removed through only the same end through which it entered the column, by evaporation.

Finally, after the solvent has been removed from the slurry that has been drawn into the column, leaving only the packing material that was in the slurry, a portion of the packing at the end of the column is optionally sintered.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a cross sectional view of a capillary column **1** the end of which is inserted into a supply of slurry **2**, which has been drawn into the capillary column.

FIG. **2** is a cross sectional view of the capillary column of FIG. **1** after evaporation of a part of the solvent from slurry **2** through the same end of the column through which the slurry originally entered the column.

FIG. **3** is a cross sectional view of the capillary column of FIG. **2** after further evaporation has taken place.

FIG. **4** is a cross sectional view of the capillary column of FIG. **3**, after complete evaporation of the solvent has taken place.

FIG. **5** is a photograph of the end of a capillary column into which a slurry of packing material has been drawn by capillary action.

FIG. **6**. is a photograph of the capillary column of FIG. **5**, after partial evaporation of the solvent in the slurry has taken place, out of the end of the column.

FIG. **7** is a photograph of the capillary column of FIG. **6**, after further evaporation has taken place.

FIG. **8** is a cross sectional view of a vessel **3** having a conical bottom **4** with a hole **5** into which capillary **1** has been inserted whereby slurry **2** is drawn into column **1** by capillary action.

DETAILED DESCRIPTION

The slurry used in accordance with the invention is a slurry of a packing material in a volatile solvent. Such slurries are prepared by mixing a packing material with a solvent.

The packing materials may be particles of a variety of shapes, such as spherical, hemispherical, "irregular" spheres, rods with aspect ratios of <5:1, fractured "chips" (i.e., shapes associated with finely ground materials), precipitated crystallites (tiny cubes, prisms, dodecahedral, etc.). Spherical or nearly spherical shapes are preferred, however, since such shapes allow for the most uniform and dense packing. The packing materials may be solid, hollow or porous such as, for example, solid, hollow or porous spheres.

Preferred packing materials are ceramic, metallic or polymeric. The ceramic materials which can be used include, for example, soda-lime glass, borosilicate glass, porous silica (silica gel) and non-porous silica. The metals which can be used include, for example, colloidal gold, colloidal silver, nickel and stainless steel. The polymeric materials which can be used include, for example, fluoropolymers, such as polyvinylidene fluoride (PVDF), fluorinated ethylene propylene (FEP); styrenics, such as polystyrene (PS) and polystyrene/divinylbenzene copolymer (PS/DVB); polyolefins such as high density linear polyethylene (HDPE), low-density linear polyethylene (LDPE) and polypropylene; polyketones, such as polyetheretherketone (PEEK); acrylics, such as polymethylmethacrylate (PMMA) and vinyls, such as divinylbenzene (DVB). Particularly preferred materials are borosilicate glass, silica (both porous silica and non-porous silica) and PS/DVB copolymer.

The particles which are used should have dimensions, i.e., diameters in the case of spheres, which are smaller than the

smallest internal dimension of the column to be used, if the column has an internal shape other than round, or smaller than the internal diameter if the column, if the column to be used has a round internal shape, and should have maximum dimensions, or diameters if spherical, of about ½ the smallest internal dimension or diameter of the columns used. In general, the largest dimensions of non-spherical particles, or the diameters of the spherical particles used, range from about 0.1 μm to about 1 mm, although a range of 0.25 μm to about 250 μm is preferred; a range of 0.5 to 30 μm being particularly preferred, a range of 1 to 5 μm being especially preferred, and a range of 2 to 4 μm being very especially preferred.

There are many solvents known to the art which can be used to form the slurry. Preferred solvents are methanol, acetone and tetrahydrdrofuran (THF), although almost any volatile solvent can be used. The solvent selected should, of course, be one that will not dissolve, swell or otherwise harm the packing material selected, although it should "wet" the surface of the packing material.

The capillary tubes which are used for the columns are those known to the art, and can, for example, be those which are generally classified as ceramics, such as borosilicate glass, fused-silica, polyimide coated fused-silica and aluminum coated fused-silica; metallic, such as stainless steel, glass lined stainless steel or silica lined stainless steel; or they can be of polymeric materials. The polymeric material which can be used include fluoropolymers, such as ethylene tetrafluoroethylene (ETFE), fluorinated ethylene propylene (FEP) and polytetrafluoroethylene(PTFE); polyolefins, such as high density linear polyethylene (HDPE), low-density linear polyethylene (LDPE) and polypropylene; polyketones, such as polyetheretherketone (PEEK) and silica-lined PEEK; acrylics, such as polymethylmethacrylate (PMMA), polyamides, such as nylon 6, nylon 11 and nylon 12; and polyimide. Preferred capillary tubing for use as capillary columns in accordance with the invention are those of polyamide-coated fused silica, stainless steel, PEEK and HDPE, although polyimide-coated fused silica is especially preferred.

The internal or external shapes of capillary columns used in the practice of this invention can take on a variety of regular geometric shapes, such as round, oval, square, rectangular, polygonal, such as pentagonal, hexagonal, and the like; or can take on irregular shapes. The term "internal shape" of the capillary columns, as used herein, has the same meaning as the "bore" of a capillary column. Particularly preferred are those columns having a round internal shape or bore.

The columns used in the practice of the invention, having round internal shapes or bores, have inside diameters in the range of from about 1 μm to about 2 mm, preferably 5 to 250 μm and particularly preferably 20 to 100 μm. Where columns having internal shapes other than round are used, their internal cross-sectional areas should be in the same range as that of a column having a round internal shape with a diameter in the range of from about 1 μm to about 2 mm, preferably that of a column having a round internal shape with a diameter 5 to 250 μm and particularly preferably that of a column having a round internal shape with a diameter 20 to 100 μm.

The columns can be of uniform internal dimensions or diameter over their entire length, such as those typically used as chromatography columns, or they can be tapered at one end, so that the internal diameter tapers to a narrow tip or needle, such as those columns used for electrospray ionization mass spectrometry (ESI-MS). The columns having tapered ends are also referred to in the art as needles. FIGS. 1–7 illustrate the ends, or tips, of the tapered-end columns, also referred to as needles. Where such tapered-end columns are used, the end or tip of the column, also referred to as the end of the needle, which is the end of the column where the packing is to be loaded, has an internal diameter ranging from about 1 μm to about 100 μm, preferably from 5 to 30 μm, particularly preferably 10 to 20 μm, especially preferred is a diameter of 15 μm. The length of the tapered portion, meaning the length of column over which the diameter tapers from the internal diameter of the untapered portion of the column to the internal diameter of the tip ranges from about 0.1 to 10 mm, preferably from 0.25 to 3 mm, especially preferably from 0.5 to 1.5 mm.

The length of the columns to be used will vary with the contemplated application, as well as the amount of additional packing, if any, which is to be used in combination with the packing of the present invention. That is to say, the packing of the present invention can be used alone, or in combination with other packings which can be added to the column before or after the present packing. Packed columns with lengths of 19 meters or more are known (U.S. Pat. No. 4,793,920), and such columns can be used in the practice of this invention, for which the length of the column used is not limited. As will be understood by those skilled in the art, however, the amount of packing that can be packed into a column using the method of this invention is limited by the capillarity between the slurry of packing material and the capillary column, which results in a specific theoretical maximum capillary height for each combination of slurry composition and capillary column composition, at given ambient conditions. The maximum capillary height for any particular application can easily be determined by simply inserting one end of the capillary column into the slurry, and observing how high into the column the slurry is drawn.

The slurry can be prepared by conventional methods, known to those skilled in the art. One such method is simple mixing, wherein a solvent is introduced into a vessel, such as a vial, beaker or a flask, together with the packing material, and the contents are then stirred. The capillary tube can then be inserted into the thus prepared slurry in the vial, beaker or flask, whereupon the slurry is drawn into the capillary tube by capillary action. The slurries according to the invention are formed with about 0.002 to 8 grams of packing per ml of solvent, preferably 0.03 to 5 grams of packing per ml of solvent, particularly preferably 0.2 to 1 gram packing per ml of solvent.

Alternatively, the slurry can be transferred from the vessel in which it was prepared into another vessel, such as a vessel having a conical bottom with a hole at the apex of the conical bottom, such as is shown in FIG. 8. One such vessel which can be used in accordance with the invention, is a common polyethylene pipette tip. In yet another embodiment of the invention, a vessel having a conical tip with a hole in the apex of the cone and a septum in the hole can be used. When using such vessels, the capillary tube is inserted into the slurry through the hole or, if the hole in the vessel is equipped with a septum, through the septum.

The time required to draw the slurry into the capillary tube by capillary action varies, depending upon a variety of factors, such as the dimensions of the packing material, i.e., the diameter of the spherical packing material, as well as the density of the slurry and the inside diameter of the capillary tube. In general, however, the slurry will be drawn into the capillary virtually instantly upon insertion of the tube into the slurry. Depending upon the extent to which it is desired

to fill the capillary tube with packing, more or less time will be required. If it is desired to pack only the tip of the capillary tube, to form a frit, for example, the desired amount of packing can be drawn in less than one second. If, on the other hand, it is desired to fill the column to the maximum achievable extent, known as the "full capillary height", several hours may be required. In general, however, the desired amount of slurry can be drawn into the capillary tube in from about 0.1 second to about 2 hours, although in most cases it will require only from about 0.1 second to about 30 minutes. The time required will, of course, vary depending upon the particular nature of the slurry and that of the capillary tube, as well as the conditions at which the filling is being conducted, such as temperature and pressure. Although it is possible to conduct the filling operation at elevated temperature and either elevated or reduced pressure, satisfactory results are generally obtained at ambient temperature and pressure.

Where no septum is used, the hole in the conical bottom of the aforedescribed vessel should be of such a size that the surface tension of the solvent in the slurry will prevent passage of slurry through the hole. Such holes will range in size from a diameter of about 0.1 mm to about 3 mm, preferably about 0.3 mm to about 1 mm.

A particular advantage of using such conical bottomed vessels is that the slurry, once placed in said vessel, can be allowed to settle, thereby forming a higher concentration of packing per ml of solvent in the bottom of the vessel, and the slurry that is drawn into the capillary tube from the vessel will thus have a higher concentration of packing than the original slurry.

A further advantage of using a conical bottomed vessel is that, as the slurry settles out in the conical bottom, a concentration gradient is formed, whereby the concentration of particles in the solvent gradually increases from the top of the vessel to the bottom. The density of the slurry that is drawn into a capillary tube inserted into the hole at the apex of the cone in the conical bottom of the vessel can then be controlled by varying how far the tube sticks up into the vessel. The closer the end of the capillary tube is to the bottom of the cone, the higher the density of the slurry drawn into the capillary tube will be.

In a particularly preferred embodiment, the slurry comprises glass microspheres as packing material in methanol as solvent, at concentration of about 0.2 grams of microspheres per ml of methanol.

In a particularly preferred embodiment of the invention, the packing material in the column is sintered, after the solvent has been removed. The packing is sintered by applying energy to it. This is done by heating the packing material with a heat source, such as hot air, by laser radiation, microwave heating or a combination of such heating means. The amount of heat applied is controlled to be sufficient to sinter the packing material, while avoiding melting it. A sufficient amount of heat must be applied so as to cause the packing material to soften. (For glass, silica or polymeric materials, the glass transition temperature must be reached.) Heating time and temperature must be sufficient to cause the particles to fuse together, but not so long or so hot as to eliminate all of the inter-particle spacing and voids. Complete melting of the packing material is, of course, to be avoided.

In the practice of this invention, the slurry is drawn into the capillary column, as described above, through capillary action. Then, the solvent that is in the slurry that has been drawn into the column is removed from the slurry through the same end of the column through which the slurry entered the column. Preferably, the solvent is removed from the slurry by evaporation. In conducting the evaporation of the solvent, the column can be allowed to stand in ambient air, as, for example may exist in a fume hood, or by passing a stream of a gas, such as air or nitrogen, over the tip of the end, to accelerate evaporation. The rate of evaporation can be controlled by such factors as the rate of gas which is passed over the tip of the end of the column, as well as the ambient temperature and the temperature of the gas that is passed over the tip of the end of the column.

Alternatively, the solvent can be removed through the end of the column by other means, such as "blotting", by holding an absorbent material, such as a filter paper or membrane, over the end of he column to contact and draw solvent out of the column.

In one embodiment of the method of the invention, a slurry that is close to the theoretical maximum density of particles suspended in a unit volume of solvent is used. In such a case, the dense slurry, when drawn into the end of the capillary tube, will remain tightly localized near the end of the capillary tube and, when the solvent is evaporated, the meniscus packs the slurry into a tight slug of material.

In yet another embodiment, the capillary tube is pre-filled with solvent, and then a portion of the solvent is permitted to evaporate, leaving an air gap at the end of the column. The end with the air gap is then inserted into the slurry, whereby the amount of slurry that is drawn into the column is limited by the air gap between the entering slurry and the pre-filled solvent. Then, the solvent, including both the pre-filled solvent and the solvent of the slurry is evaporated. As the solvent evaporates, the meniscus movement packs the packing material into a tight porous plug at the end of the tube.

Referring now to the drawings, FIG. **1** shows the end tip of a tapered-end capillary column **1**, also referred to as a needle, of the present invention. As shown, the capillary tube is inserted into a reservoir of slurry **2**, comprising packing spheres **6** dispersed in a solvent, and the slurry has been drawn into the tube by capillary action.

FIG. **2** shows the capillary tube end-tip of FIG. **1**, after partial evaporation of the solvent out of the end-tip. As is illustrated, the meniscus of the surface of the slurry inside the column and furthest removed from the end tip of the column has moved towards the end tip, as solvent is evaporated from the end tip. As also illustrated, and this is an important feature of the present invention, the meniscus, as it moves towards the end-tip, brings with it a concentration of the packing material, so that as the solvent is evaporated from the end-tip, the slurry concentrates itself towards the end-tip. This is entirely surprising, as the packing material would have been expected to "precipitate out" of the solvent, and be left behind in a dry state, as the volume of solvent shrinks towards the end tip. It was not to be expected that the moving meniscus would actually carry the packing material towards the end-tip, thereby concentrating the packing material in the direction of the end tip. It has now been discovered that as the liquid solvent evaporates, the meniscus traveling towards the end of the tube collects and gently forces the packing material towards the end of the tube. The surface tension of the liquid/air (or liquid/nitrogen) interface at the end of the tube is sufficient to hold the packing material in place in the slurry against this gentle moving force. The tube may optionally be vibrated and/or slowly rotated on its axis during the evaporation process to promote dense packing of the packing material.

FIG. **5** is an actual photograph of a capillary tube such as that illustrated in FIG. **2**.

FIG. 3 illustrates the column of FIG. 2 after further evaporation has taken place, whereby the concentration of the packing material has been further increased and moved closer to the end-tip.

FIG. 6 is an actual photograph of a capillary tube such as that illustrated in FIG. 3.

FIG. 4 illustrates the column of FIG. 3 after all of the solvent has been evaporated. The packing material illustrated in FIG. 4, with the solvent removed, is ready for sintering.

FIG. 7 is an actual photograph of a capillary tube such as that illustrated in FIG. 4.

The method of the present invention can be better understood by reference to the following example, but is not limited thereby.

EXAMPLE 1

A 10 cm long piece of 360 μm outside diameter (OD), 75 μm inside diameter (ID) polyimide coated fused silica tubing (Polymicro Technologies, Phoenix, Ariz.) was mounted in a commercial laser-heated micropipette puller (Sutter Instruments, Novato, Calif.) and drawn down into two sharp needles so that the internal diameter at the needle end was reduced to 15 μm.

A slurry of 5 μm diameter solid glass microspheres was prepared by mixing 0.1 gram of the spheres with 0.5 milliliters of 100% methanol in a small (1 ml) glass vial. The mixture was stirred thoroughly for 5 minutes, ultrasonicated for 5 minutes, and then allowed to settle for 2 hours. After removing the excess solvent above the slurry, which had settled to the bottom of the vial, approximately 10 μl of slurry was transferred to a polyethylene vessel (a commercial polyethylene pipette tip) possessing a conical bottom having a 0.5 mm hole at it's apex. The slurry was again allowed to settle for approximately 5 minutes, while holding the pipette tip in the vertical position.

Both the slurry-containing vessel and the silica needle were mounted horizontally on the stage of a standard light microscope and viewed at 100-x magnification. The microscope stage had an additional translation stage that allowed the silica needle to be moved into the hole of the pipette tip. By moving the tip into brief contact (≈0.5 seconds), with the slurry through the hole, approximately 75–100 microspheres were transferred into the silica needle.

The needle was allowed to rest on the stage of the microscope for 5 minutes while the methanol completely evaporated from the tip of the needle. During this time, the silica was packed into place by the movement of the meniscus.

The needle was transferred to a device holding a platinum foil heating element with a 3 mm "trough" filament, 3 mm wide (Sutter Instruments filament number FT330B). The end of the needle containing the packed spheres was centered in the filament. The filament was energized for 12 seconds with a heat output of 20.1 watts, to sinter the microspheres.

EXAMPLE 2

The procedure of Example 1 was repeated, except that a needle fabricated from 50 μm ID fused silica tubing pulled down to an 8 μm ID tip was used. Approximately 25–50 spheres were transferred into the end of the silica needle. For sintering, the heat output of the filament device was reduced to 19.4 watts.

EXAMPLE 3

The procedure of Example 1 was repeated, but a 10 cm piece of 50 μm ID, 360 μm OD polyimide coated, fused silica tubing was cut with a clean square end-face. The slurry was prepared as in Example 1, but 10 μm highly porous spheres of poly (styrene-divinylbenzene(Poros®, Perspective Biosystems 10-R2) were substituted for the glass microspheres. The column was loaded as described in Example 1. The packing was sintered in the same apparatus to form a frit, but the heating conditions were 3 seconds at 6.3 watts

EXAMPLE 4

A 25-cm length of 75 μm ID tubing was cleaved to have a flat end-face.

A slurry was prepared as in Example 1. After settling, 100 μl of slurry was transferred to a polyethylene pipette tip. The tip was held in the vertical position and the slurry allowed to settle for 15 minutes. Settling time was monitored with a light microscope to ensure that >95% of the material had settled out to the bottom of the tip.

While left in the vertical position, the silica capillary tube was inserted approximately 50 μm into the hole at the bottom of the pipette tip. The dense (nearly opaque) slurry filled the silica tube by capillary action. The tube was left in contact with the slurry until 15 cm of the tube was filled.

Upon removal, the end of the silica tube was brought into contact with a Nylon filter pad (Milipore Corp.), and mounted horizontally on the stage of a light microscope. The movement of the meniscus and packing of the column was monitored for approximately 45 minutes. When evaporation appeared to be complete, the Nylon filter paper was removed and packing material at the end of the tube was sintered into a frit by heating in the heating device described in Example 1, with a filament output of 25 watts for 12 seconds.

EXAMPLE 5

The procedure of Example 4 was repeated, except that the filling was accomplished with the tube in the horizontal position. A column with 10 cm of packed material was thereby fabricated.

The invention and its advantages are readily understood from the foregoing description. It is apparent that various changes can be made in the process without departing from the spirit and scope of the invention. The process as herein presented, is merely illustrative of preferred embodiments of the invention, and not a limitation thereof.

I claim:

1. A method for forming a frit in the end of a tapered capillary tube useful as a source of ionized droplets for electrospray ionization mass spectrometry, which comprises selecting a hollow capillary tube having a tapered end in which a frit is to be formed, forming a slurry of a sinterable material in a volatile solvent, inserting said tapered end into said slurry an allowing said slurry to be drawn into said tapered end by capillary action, withdrawing said tip from said slurry, allowing said solvent to evaporate, leaving behind the sinterable material that was drawn into said tapered end as a part of said slurry, and applying a source of energy to said sinterable material to sinter it.

2. The method of claim 1, wherein said sinterable material is glass, silica, metal or polymer.

3. The method of claim 2, wherein said sinterable material is glass, in the form of spheres having a diameter of from about 0.25 μm to about 250 μm.

4. The method of claim 2, wherein said sinterable material is silica, in the form of spheres having a diameter of from about 0.25 μm to about 250 μm.

5. The method of claim 2, wherein said sinterable material is glass, in the form of spheres having a diameter of from about 0.25 μm to about 250 μm.

* * * * *

US005395521A

United States Patent [19]

Jagadeeswaran

[11] Patent Number: 5,395,521

[45] Date of Patent: Mar. 7, 1995

[54] **AUTOMATED COLUMN EQUILIBRATION, COLUMN LOADING, COLUMN WASHING AND COLUMN ELUTION**

[75] Inventor: **Pudur Jagadeeswaran**, San Antonio, Tex.

[73] Assignee: **Board of Regents, The University of Texas System**, Austin, Tex.

[21] Appl. No.: **707,880**

[22] Filed: **May 31, 1991**

[51] **Int. Cl.**[6] **B01D 15/08**

[52] **U.S. Cl.** **210/198.2**; 210/143; 210/656; 96/103

[58] **Field of Search** 210/635, 656, 659, 198.2, 210/143; 55/67, 386; 422/70; 436/161, 162; 96/101, 102, 103; 95/82

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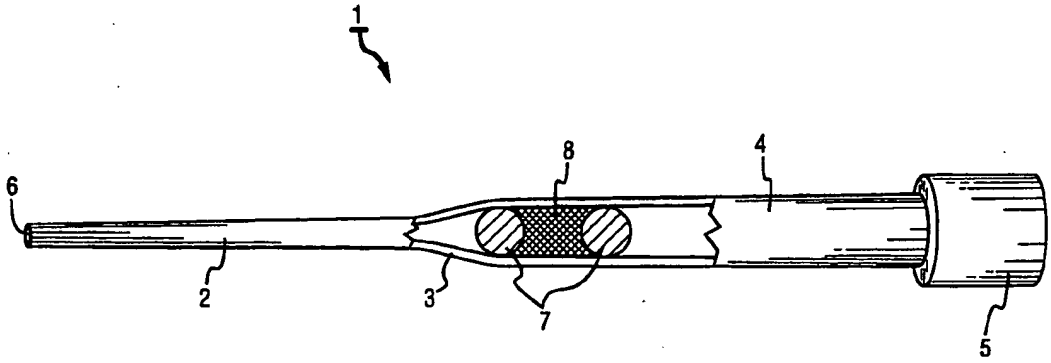
Primary Examiner—Ernest G. Therkorn

Attorney, Agent, or Firm—Arnold, White & Durkee

[57] **ABSTRACT**

Method and apparatus for automatic chromatographic column operations, including column equilibration, column loading, column washing, and column elution. A novel pipette tip column is shown which is packed with a binding material sandwiched between two permeable spheres.

22 Claims, 1 Drawing Sheet



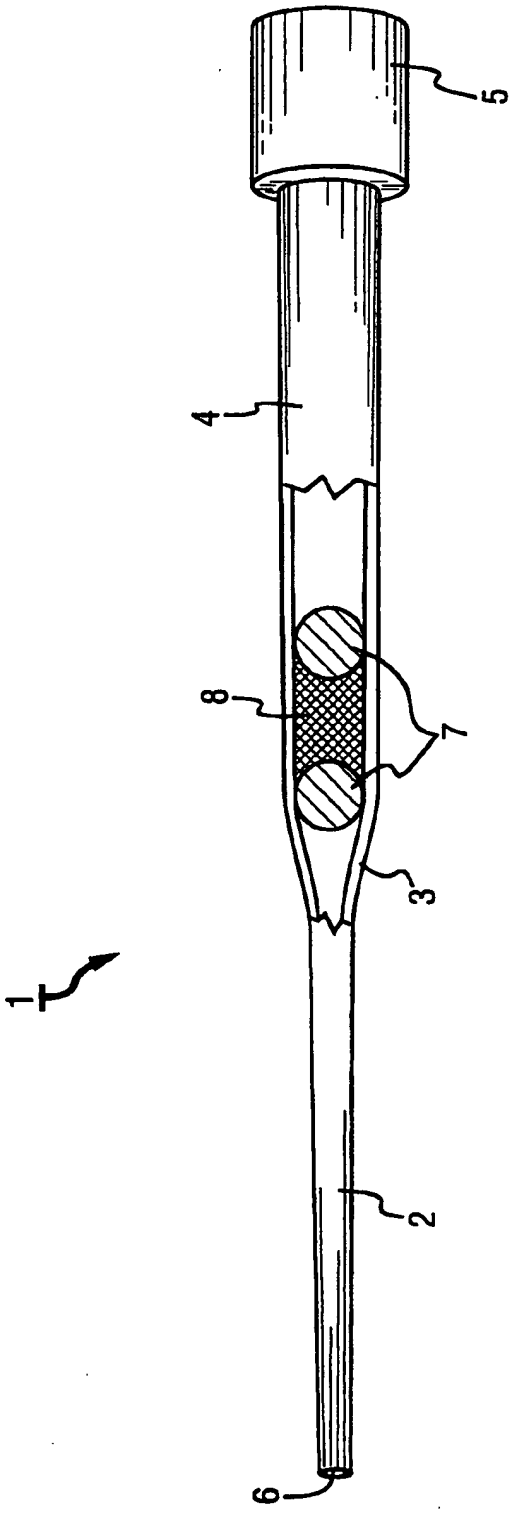


FIG. 1

AUTOMATED COLUMN EQUILIBRATION, COLUMN LOADING, COLUMN WASHING AND COLUMN ELUTION

BACKGROUND OF THE INVENTION

(1) Field of the Invention

This invention relates generally to automated chromatographic column operations, including column equilibration, column loading, column washing, and column elution. More particularly, the invention resides in a system in which a chromatographic column is moved in an automatic programmed sequence from one fluid source or discharge point to another. The invention is especially directed at the adaptation of an automatic pipetting system to an automatic chromatographic system.

(2) Description of the Prior Art

Many laboratory procedures rely upon column chromatographic methods. These procedures include the purification of DNA and oligonucleotides, preparation of poly A RNA and high molecular weight genomic DNA, and the recently developed column-based solid phase method of performing Maxam-Gilbert sequencing reactions. See "Use of Reverse-Phase Chromatography in the Maxam-Gilbert Method of DNA Sequencing: A Step Toward Automation," Jagadeeswaran, P. and Kaul, R. K., *Gene Analysis Techniques*, 3:79–85 (1986). In a multi-step procedure such as Maxam-Gilbert sequencing reactions, using commercially available columns and running them is a labor intensive process.

Robotic pipetting machines are well known in the art. For instance, the Biomek ® 1000 Automated Laboratory Workstation (Beckman; 2500 Harbor Boulevard, Fullerton, Calif.) comprises a robotic arm which can hold a pipette tool and is capable of vertical and horizontal (forward and backward) movement. The Biomek ® 1000 includes a platform which carries pipette tools, and separate storage ports which are holding places for pipette tips and fluids. The Biomek ® 1000 can be programmed to move a pipette tool and connect to a pipette tip in such a manner as to enable the tool to intake and discharge liquid through the pipette tip. Typically, the Biomek ® 1000 is programmed to move a pipette tool to a pipette storage location, connect the tool to a pipette tip, move to an instructed port which holds a liquid, pick up an instructed amount of liquid, and deliver this liquid to another location.

SUMMARY OF THE INVENTION

A general object of this invention is to provide an improved chromatographic system capable of automatic column equilibration, column loading, column washing, and column elution. In one embodiment of this invention an automatic pipetting apparatus is adapted to provide such a chromatographic system. The pipette tips used in the automatic pipetting apparatus are modified to perform as chromatographic columns, and to be transferred by the automatic pipetting machine from one position to another.

In a general aspect, the present invention comprises a vertically disposed column capable of holding a charge of a binding material or chromatographic medium; a plurality of column stations including a storage position, at least one separate fluid dispensing station, at least one separate receptacle station; and an automatic column handler programmable to automatically engage the column, move the column sequentially between a plurality of said stations, receive fluids at the dispensing stations, and discharge fluids at the receptacle stations. In a preferred form, the binding material holders employed with the invention are miniature columns which are necked or otherwise configured to receive a first permeable plug above a charge of chromatographic medium binding material within the column, and a second permeable plug below the charge. The plugs are configured to retain a charge of chromatographic medium between them, and to enable fluids to permeate through the plugs. At least one of the plugs is removable from the column, so as to enable the column to be charged with a chromatographic medium. Preferred plugs are unitary elastomeric members which are capable of being received and held by recesses, grooves, necked sections, or other internal structural seat-like features of the column. Thus, it is especially preferred that a plug be readily forced to seat within such a structural feature, and also be readily recovered. When seated within a column, it is desired that the plug seal against the internal wall of the column such that fluid will permeate through the body of the plug in preference to its periphery.

It is also preferred that the column for the chromatographic medium be a pipette-like member having a relatively long diameter central section and an extended necked section of smaller diameter. Indeed, an especially preferred column is a member resembling a pipette tip such as are employed with conventional automatic pipetting machines such as the Biomek ® 1000. These machines are equipped and programmed to move pipette tips from one station to another, where the automatic pipetting machine then typically aspirates or discharges fluids through the pipette tips. In adapting the present invention to such apparatus, a pipette-shaped column is charged with a chromatographic medium binding material, and is moved from one station to another. The fluid dispensing and receptacle stations may be prepared by modifying existing fluid reservoir ports on the Biomek ® 1000.

The Biomek ® 1000 pipette tips are more preferably modified in accordance with the invention to become miniature chromatographic columns by preferably placing a porous permeable polyethylene sphere into the large end of the pipette tip and then pushing the sphere into location just above the "neck" of the pipette tip. The sphere is conveniently pushed into location with a steel rod. The sphere is then topped with a specific amount of a selected resin, chromatographic medium, or other binding material. A second porous permeable polyethylene sphere is then placed on top of the medium and firmly packed with the steel rod. The spheres prevent the binding material from moving during fluid intake and discharge operations performed by an automatic pipetting machine. Once correctly packed, these pipette tips became miniature chromatographic columns and are herein referred to as "columns."

Once a pipette tip is improved as described above, it is possible to program an automated pipetting apparatus to connect to a column, and then automatically equilibrate, load, wash, and elute the column. This procedure is performed by preparing an automatic pipetting apparatus with a packed column, a charging fluid source, an elution fluid source, a charging fluid discard location, and an elution fluid storage location. The packed column may be one of a plurality of such columns stored systematically in a suitable column storage facility. As

used in this specification, "charging fluid" means any fluid used to equilibrate, load, or wash the column. In some instances the charging fluid may also include a fluid used to elute the column, although preferably the eluting step takes place after the charging step. "Fluid" means any gas or liquid.

Once prepared, the apparatus is programmed and operated to perform the following steps: move a pipette handling tool to the packed column storage; connect to a packed column so as to allow intake and discharge of fluid through the column; move to the charging fluid source; charge the column by intaking charging fluid into the column; move the column to the charging fluid discard location; and discharge the charging fluid. Successive selected fluids may be drawn into the column and discharged to successively equilibrate, load, and wash the packed column. In this manner the column is automatically "charged."

Once charged, the packed column may be eluted by moving the column to the elution fluid source and intaking elution fluid into the column. In this manner the column is "eluted." The column may then be moved to the elution fluid storage and the elution fluid discharged into the elution liquid storage. In this manner the elution fluid is recovered in a separate location from the other discharged fluids. The operation may then be repeated using a new column.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a schematic view of a pipette tip column loaded with a chromatographic medium and porous permeable polyethylene spheres.

DESCRIPTION OF PREFERRED EMBODIMENTS

A Biomek® 1000 Automated Workstation (Beckman; Fullerton, Calif.) was used to implement the automatic pipetting system of this invention because it comprises a programmable robotic arm connected to a pipette tool. The pipette "tool" is the section of the Biomek® 1000 apparatus that is connectable to the pipette tip. The arm and tool are capable of vertical and horizontal (forward and backward) movement. The Biomek® 1000 is programmable and capable, among other things, of automatically moving a pipette from one location to a second location, intaking fluids into the pipette, moving the pipette to a third location, and then discharging fluid from the pipette. The pipette tool is specially designed to connect to pipette tips.

The Biomek® 1000 includes a male pipette tool which is connectable in a relatively airtight manner to the female large end of a Biomek® 1000 pipette tip. The Biomek® 1000 pipette tool intakes fluids into a pipette tip by connecting itself to the pipette tip and exerting a vacuum force on the large end of the pipette tip, thereby sucking fluids through the small end of the pipette tip and into the pipette tip. The Biomek® 1000 can be programmed to intake specific amounts of fluid into a pipette tip. Fluids are discharged from a pipette tip when the tool relaxes the vacuum force. If desired, however, the tool can be programmed to provide positive discharge air pressure to force fluid from a pipette tip.

The Biomek® 1000 may be connected to a computer to control the apparatus. In the preferred embodiments, an IBM PS/2 computer, and a commercial liquid handling program (Genesis Software Version 2.0; by Beckman) were used to control the apparatus.

In implementing the invention, radiolabeled DNA was used and binding was monitored by a liquid scintillation counter. Binding efficiencies were compared by counting the DNA solution before and after loading, counting the columns after binding, and counting the eluent. The DNA was obtained from/prepared by polymerase chain reaction using terminally labeled oligonucleotides. The liquid scintillation counter used was a Searly Analytical Inc. ISOCAP/300 6872. Appropriate liquid reagents were used for column equilibration, column loading, column washing, and column elution according to manufacturers recommendations. "Appropriate" in this context means those reagents that would adequately equilibrate, load, wash, or elute the column as desired by the practitioner. Depending on the materials examined, these reagents will vary as is well known in the art. In the particular experiments conducted herein, the equilibrating, loading, and washing fluid was 0.2M NaCl in TE (10 mM Tris-HCl, pH 7.2, 1 mM EDTA) as per the NACS PREPAC Instruction Manual, p. 12 (Bethesda Research Laboratories Life Technologies, Inc.). The eluting fluid was 2.0M NaCl in TE as described in the same NACS PREPAC Instruction Manual.

Tubular Biomek® 1000 (250 milliliter ("ml")) pipettes (Beckman catalog No. 373685) were packed to form columns for the automatic pipetting apparatus. "Packed" in the context of the invention means inserted in the pipette tip in such a manner as to resist dislodging when other materials are intaken or discharged through the pipette tip and the permeable porous material. "Binding materials" means any chemical or other chromatographic medium that will react, absorb, adsorb, or interact in some desired fashion with other materials that may flow through the pipette.

FIGURE 1 shows a packed Biomek® 1000 pipette (column) 1 which comprises a large end 5, a middle section 4 connected to the large end 5, a neck section 3 connected to the middle section 4, a tube body section 2 connected to the neck section 3, and a small end 6 connected to the tube body section 2. The Biomek® 1000 column is packed with two porous polyethylene spheres 7 and binding material 8. Binding materials 8 actually used included NACS-52 (BRL, Gaithersburg, Md.) and C18 resin (Waters, Division of Millipore, Milford, Mass.). It will be recognized that many other binding materials may be used such as well known in the art. If the liquid flowrates are critical, it may be possible to vary the bead or granule size of the binding material to allow the liquid to flow more easily through the binding material. "Porous" in the context of this application means relatively permeable to liquids but relatively impermeable to solids.

The spheres 7 may also be made of many suitable porous materials such as polyethylene. It is understood that the spheres 7 are preferably made of a soft material that will mold itself to the inside of a pipette and provide a snug fit therein. It is also understood that the word "sphere" as used in the context of this application only means objects that are roughly spherical. Irregularly shaped porous materials that will mold themselves to the shape of the interior of the pipette and provide a snug fit therein are included within this definition.

Glass wool may also be used in place of the polyethylene spheres 7, however polyethylene spheres are preferred. When glass wool was packed in a column and then topped with binding material 8, the glass wool and/or binding material 8 tended to dislodge during the

intake or discharge operations of the pipetting apparatus, thus interfering with the binding or chromatographic process. When glass wool was packed both above and below the binding material 8 in the pipette 1, the pipette tool experienced difficulty in suctioning fluid into the pipette 1, and thus binding efficiency was decreased.

Porous polyethylene sheets were also cut to fit in various locations within the pipette 1 in place of the polyethylene spheres 7, however results were similar to those achieved with the glass wool. Binding efficiencies with the polyethylene sheets varied because of the non-uniformity of the packing as well as the channeling effect of improperly packed columns.

The position of the porous polyethylene spheres 7 with respect to the small end 6 of the column 1 was altered and the amount of binding material 8 was varied in different experiments. It was discovered that a preferred location of the polyethylene spheres 7 and binding material 8 was obtained by placing the polyethylene sphere closest to the small end 6 in the middle section 4 and adjacent to the neck 3.

The packing of the pipettes 1 with porous polyethylene spheres 7 required only simple tools such as forceps, a steel rod, and a scoop of known dimension. The forceps was used to place the polyethylene sphere 7 in the pipette 1. Because the pipette 1 is wedge-shaped, the sphere 7 would not pass beyond a certain point with gravity force alone. A steel rod was used to push the sphere 7 to the middle section 4 adjacent to the neck section 3. A scoop was used to add the binding material 8 on top of the sphere 7, and a second sphere 7 was then placed on top of the binding material 8. The porous polyethylene spheres 7 were commercially available 5/32 inch NEN balls from DuPont NEN Products (Boston, Mass.).

It is possible to prepare a column without using the second polyethylene sphere 7. When columns prepared in this manner were used, the Biomek ® 1000 occasionally suctioned up liquid and binding material into the pipette tool. In such situations, binding of the DNA to the binding material was relatively efficient, however the results using these columns were not highly reproducible.

The most preferred column was obtained by packing the binding material between two 5/32 inch porous polyethylene spheres 7. Using these columns, the Biomek ® 1000 was able to intake the fluids and the binding results were efficient and reproducible. These columns were packed snugly enough so that intake and discharge forces of the Biomek ® 1000 did not loosen the column. It was noted that some 96 tips could be manually packed in about 20 minutes. This packing procedure proved to be useful not only for packing columns to be used with the Biomek ® 1000, but also for cutting the expense involved in generating any number of disposable columns.

The Biomek ® 1000 has limited available space in its fluid reservoirs, and hence it was necessary to modify these reservoirs to prepare a charging fluid discard location that would not overflow. To modify the reservoir, a vacuum suction apparatus was attached to one of the Biomek ® 1000 reservoirs to allow fluids that are discarded there to be removed quickly. To attach the vacuum suction apparatus, a hole was drilled in the center of either the 37 ml (Beckman catalog No. 373690) reservoir or the 17 ml (Beckman catalog No. 373691) reservoir, a pipette tip was pushed inside, and flexible tubing was attached from the vacuum suction apparatus to the pipette tip.

The Biomek ® 1000 was programmed in a specific embodiment to use the pipette tool to perform the following steps:

(1) move to a packed column in a column storage area and connect to the column so as to allow the pipetting apparatus to intake and discharge fluid through the packed column;

(2) move the column to a equilibration fluid source reservoir and intake a specified amount of equilibration fluid;

(3) move the column to the fluid discard location and discharge the equilibration fluid at the location;

(4) move the column to the loading fluid source reservoir and intake a specified amount of loading fluid;

(5) move the column to the fluid discard location and discharge the loading fluid at the location;

(6) move the column to a washing fluid source reservoir and intake a specified amount of washing fluid;

(7) move the column to a fluid discard location and discharge the washing fluid at the location;

(8) move the column to the elution fluid source reservoir and intake a specified amount of elution fluid; and

(9) move the column to elution fluid storage reservoir and discharge the elution fluid into the elution fluid storage reservoir.

The "fluid discard location" may be one location, or it may be several different locations.

Preferred results were achieved when the fluid intake processes were repeated at least once with each fluid prior to continuing on with the next step of the procedure. For instance, during the elution fluid intake process, the column was moved to the elution fluid source, the elution fluid was intaken and discharged (with the discharged elution fluid being returned immediately to the elution fluid source), and then the elution fluid was again intaken prior to moving the column to the elution storage reservoir. Similarly, the loading fluid intake process may be repeated in like manner to obtain preferred results. Alternately, the fluid intake processes may be repeated without returning the fluid immediately to the source. For instance, for the equilibration fluid intake process, steps (2) and (3), as outlined above, may be simply repeated prior to continuing on with step (5), etc.

It is anticipated that the present invention will help markedly to automate the solid phase Maxim-Gilbert sequence reactions. Moreover, other binding materials may be packed in these columns, thus automating other column applications, such as poly-A RNA preparation, avidin columns, Sephadex resin columns, and dyna beads columns.

Further modifications and alternative embodiments of various aspects of the invention will be apparent to those skilled in the art in view of this description. Accordingly, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the general manner of carrying out the invention. It is to be understood that the forms of the invention shown and described herein are to be taken as the presently preferred embodiments. Elements and materials may be substituted for those illustrated and described herein, parts and processes may be reversed, and certain features of the invention may be utilized independently, all as would be apparent to one skilled in the art after having the benefit of this description of the invention. Changes may be made in the elements de-

scribed herein or the steps or the sequence of steps of the methods described herein without departing from the spirit and scope of the invention as described in the following claims.

What is claimed is:

1. An apparatus adapted to perform chromatographic analysis and capable of column equilibration, column loading, column washing, and column elution, comprising a chromatographic column with a tapered tip and which is adapted at a terminal end with a permeable plug capable of holding a charge of binding material; a source of binding material; a plurality of column chromatographic stations including at least one separate fluid dispensing station and at least one separate receptacle station; and an automatic column handler programmable to automatically engage the column in a substantially upright position so that a bottom portion of the column is proximate the tapered tip during use, move the substantially upright column sequentially between a plurality of said stations during use, receive fluid upwardly through the tapered tip into the bottom portion of the column at each dispensing station during use, and discharge fluid downwardly from the bottom portion and through the tapered tip of the column at each receptacle station during use.

2. The apparatus of claim 1 wherein the column comprises a tube containing permeable plugs configured to retain a charge of binding material between the plugs.

3. The apparatus of claim 2 wherein at least one of the plugs is removable from the column, so as to enable the column to be charged and decharged with the binding material.

4. The apparatus of claim 2 wherein the plugs are elastomeric members capable of being received within the column and held in position by internal structural features of the column.

5. The apparatus of claim 2 wherein the plugs seal against the internal wall of the column such that fluid will permeate through the body of the plug.

6. The apparatus of claim 2 wherein the plugs are spherically shaped.

7. The apparatus of claim 1 wherein the column is a pipette tip-like member having a relatively long central section with an inside diameter, and an extended necked section with an inside diameter smaller than the inside diameter of the central section.

8. The apparatus of claim 1 wherein at least one dispensing station comprises a charging fluid reservoir.

9. The apparatus of claim 8 wherein at least one charging fluid is an equilibrating fluid and the automatic column handler is programmed to equilibrate the column with the equilibrating fluid.

10. The apparatus of claim 9 wherein at least one dispensing station is an elution fluid reservoir and the automatic column handler is programmed to elute the column with elution fluid from the elution fluid reservoir.

11. The apparatus of claim 8 wherein at least one charging fluid is a loading charging fluid and the automatic column handler is programmed to load the column with the loading charging fluid.

12. The apparatus of claim 11 wherein at least one dispensing station is an elution fluid reservoir and the automatic column handler is programmed to elute the column with elution fluid from the elution fluid reservoir.

13. The apparatus of claim 8 wherein at least one charging fluid is a washing charging fluid and the automatic column handler is programmed to wash the column with the washing charging fluid.

14. The apparatus of claim 13 wherein at least one dispensing station is an elution fluid reservoir and the automatic column handler is programmed to elute the column with elution fluid from the elution fluid reservoir.

15. The apparatus of claim 1 wherein at least one dispensing station comprises an eluting fluid reservoir.

16. The apparatus of claim 1 wherein at least one dispensing station is an elution fluid reservoir and the automatic column handler is programmed to elute the column with elution fluid from the elution fluid reservoir.

17. The apparatus of claim 1 wherein at least one receptacle station comprises a charging fluid discard location.

18. The apparatus of claim 1 which further comprises a vacuum source connected to at least one receptacle station such that liquid can be removed by vacuum force from a bottom of a receptacle station during use.

19. The apparatus of claim 1 wherein at least one receptacle station comprises an elution fluid storage location.

20. An apparatus adapted to perform chromatographic analysis, comprising:

a chromatographic column with a tapered tip which is adapted at a terminal end with a permeable plug capable of holding a charge of binding material;

a source of binding material;

a plurality of column stations including at least one loading fluid dispensing station, at least one charging fluid dispensing station, at least one equilibrating fluid dispensing station, at least one eluting fluid dispensing station, and at least one receptacle station; and

an automatic column handler programmable to automatically engage the column in a substantially upright position so that the column has a bottom portion proximate the tapered tip, move the substantially upright column sequentially between a plurality of the dispensing and receptacle stations to load, charge, equilibrate, and elute the column during use such that fluid is received upwardly through the tapered tip into the bottom portion of the column at each dispensing station during use, and such that fluid is discharged downwardly from the bottom portion and through the tapered tip of the column at each receptacle station during use.

21. The apparatus of claim 20 wherein the chromatographic column comprises a permeable plug positioned at each end of the binding material.

22. The apparatus of claim 21 wherein the permeable plugs are substantially spherical in shape.

* * * * *

United States Patent [19]

Donald

[11] **Patent Number: 4,787,971**

[45] **Date of Patent: Nov. 29, 1988**

[54] **MINIATURIZED COLUMN CHROMATOGRAPHY SEPARATION APPARATUS AND METHOD OF ASSAYING BIOMOLECULES EMPLOYING THE SAME**

[76] Inventor: **Alan Donald**, 3330 Second Ave., San Diego, Calif. 92103

[21] Appl. No.: **6,120**

[22] Filed: **Jan. 23, 1987**

[51] **Int. Cl.**[4] **B01D 15/08**

[52] **U.S. Cl.** **210/198.2**; 210/657; 422/70; 422/101; 436/178

[58] **Field of Search** 422/60, 69, 70, 101, 422/102; 436/161, 177, 178; 210/656, 198.2, 657, 282

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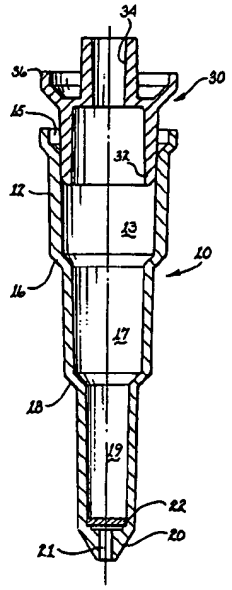
Primary Examiner—Ernest G. Therkorn

Attorney, Agent, or Firm—David G. Rosenbaum; Harry M. Weiss

[57] **ABSTRACT**

The present invention is directed to providing a column chromatography apparatus and assay method employing the same which is readily adaptable for rapid laboratory separation of chemicals, in particular, organic, inorganic and biochemicals and biomolecules from an eluent fluid, such as a body fluid. In particular, it has been determined that a miniaturized column chromatography apparatus having a multi-stage separatory tube, an associated eluent tube, an adapter and a vented-cap and a non-vented cap is particularly well suited to the rapid assays yielding accurate results which are required in the analytical laboratory or clinical setting.

24 Claims, 2 Drawing Sheets



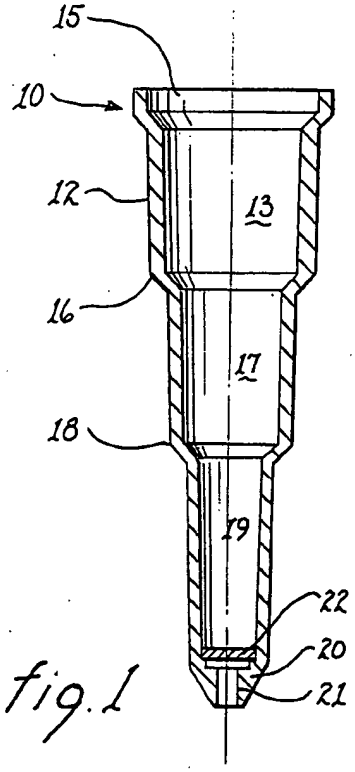


fig. 1

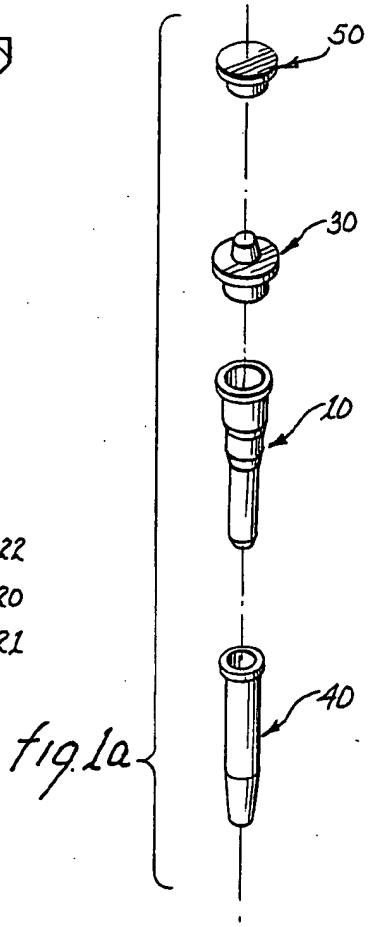


fig. 1a

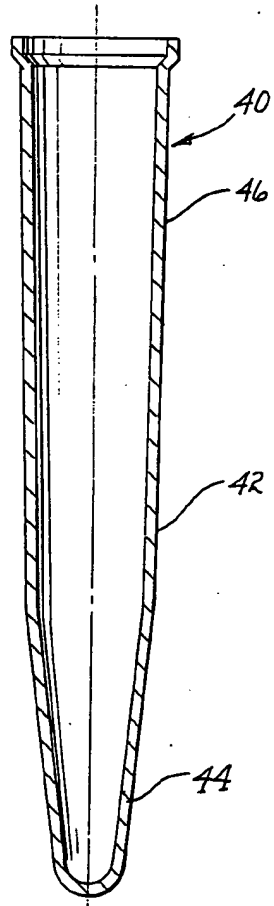


fig. 2

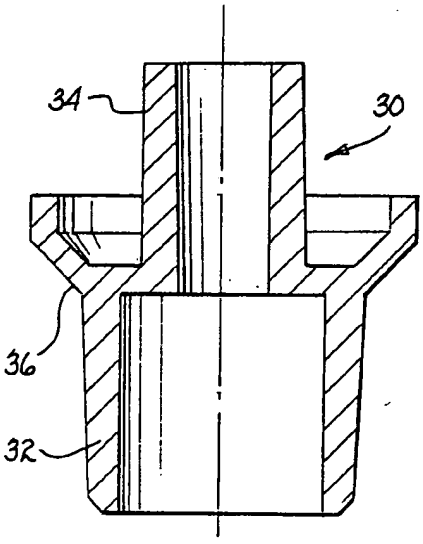


fig. 3

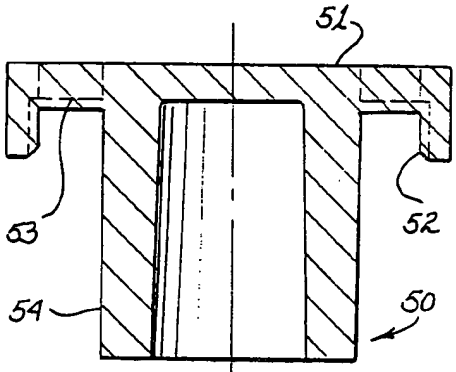


fig. 4

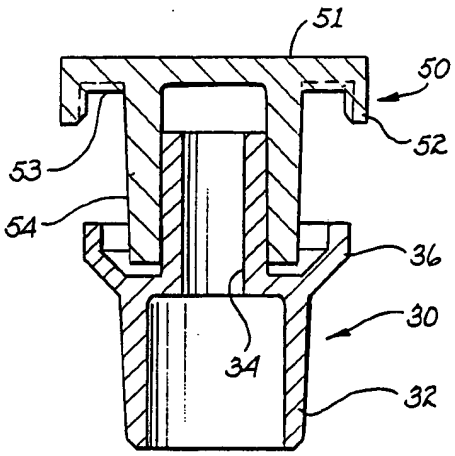


fig. 5

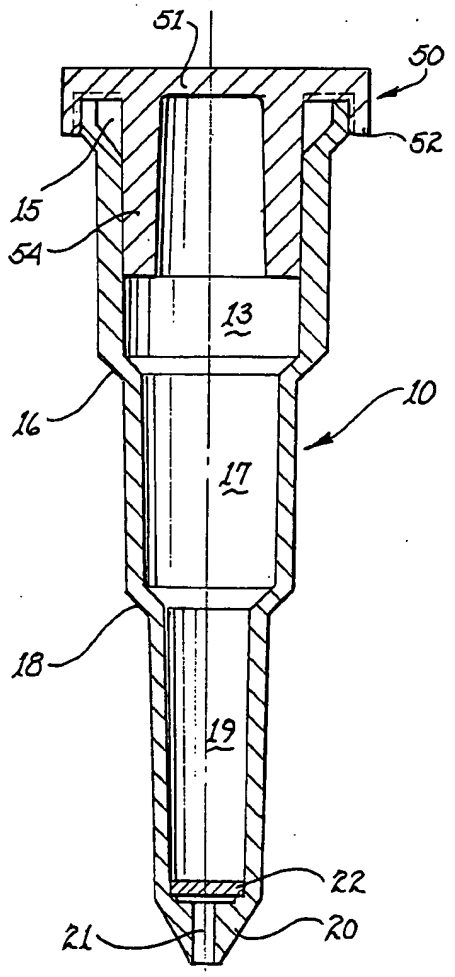


fig. 6

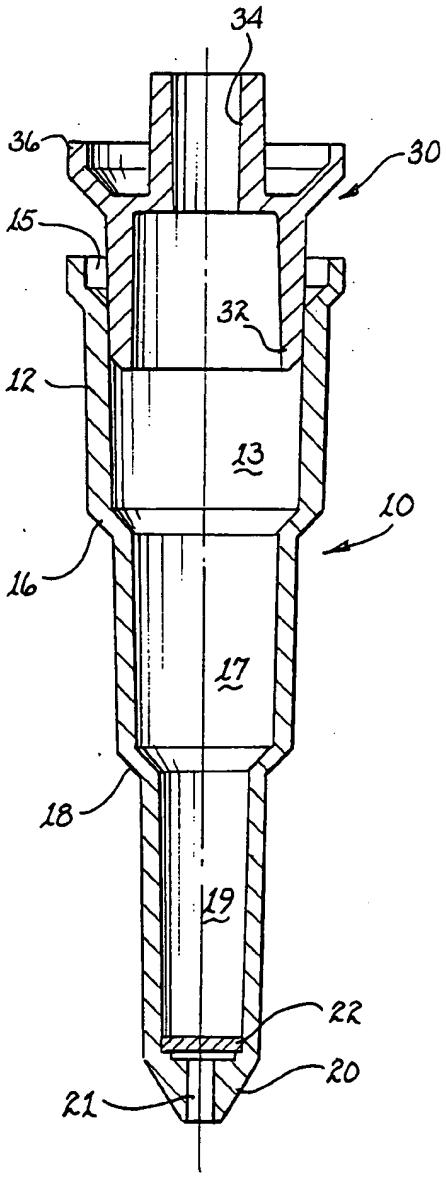


fig. 7

MINIATURIZED COLUMN CHROMATOGRAPHY SEPARATION APPARATUS AND METHOD OF ASSAYING BIOMOLECULES EMPLOYING THE SAME

BACKGROUND OF THE INVENTION

This invention relates generally to an apparatus for separation of liquid compounds by column chromatography. More particularly, the present invention relates to a miniaturized chromatography column for the rapid separation of organic chemicals, inorganic chemicals, biochemicals and biomolecules by liquid phase chromatography.

It has been found that traditional column chromatography apparatus are not easily adapted for the rapid separation of biomolecules in a laboratory setting. Where there is a need for separation of compounds from a body fluid, such as blood or urine, a laboratory technician must set up individual columns for each type of separation to be done. The process of setting up a column consists of selecting the appropriate stationary phase medium specific to the biochemical or body fluids to be separated and packing the column with the appropriate stationary phase medium. After setting up the conventional column chromatography apparatus, the technician is then able to introduce the eluent into the column and collect the eluate, then recover the separated biochemical from the stationary phase media by appropriate washings.

Conventional column chromatography apparatus provide inherently slow separations due to the length of the column or the quantity of biochemical to be separated. Numerous methods of accelerating the separations have been developed, such as use of a pump to forcedly move the eluent through the column or plungers to maintain a pressure gradient on the eluent. None of the conventional column chromatography apparatus, however, are well adapted for diagnostic, analytical or other laboratory procedures where rapid separations of biochemicals or biomolecules from body fluids are required.

Conventional column chromatography apparatus consist of glass buret-like tubes which are packed with a separatory medium. The size and length of these conventional apparatus make them unsuitable for use in rapid separations with high recovery yields. Further, conventional chromatography apparatus require the repetitive transfer of the eluate or subsequent washes to a multitude of vessels. The necessity for the use of many vessels to collect the eluate and wash increases the likelihood of contamination in the separated biochemical and, therefore, increases the likelihood of erroneous analytical or diagnostic results. Finally, conventional chromatography apparatus are typically capable of single-stage separations whereby only one type of eluent or one type of separation media may be employed. It has been found desirable to provide a miniaturized column chromatography apparatus which will meet the foregoing deficiencies commonly found with conventional column chromatography apparatus. The present invention, therefore, is directed to providing a column chromatography apparatus which is readily adaptable for rapid laboratory separation of biochemicals and biomolecules from an eluent fluid, such as a body fluid. In particular, it has been determined that a miniaturized column chromatography apparatus having a multi-stage separatory tube, an associated eluent tube, a vented-cap and a non-vented cap wherein the entire apparatus is sized to be usable with a common laboratory centrifuge or, if necessary, an ultracentrifuge and is adapted to fit conventional test tubes, holder racks, mini-vials, syringes and other laboratory equipment. By providing a column chromatography apparatus configured in this manner, a laboratory technician is able to rapidly separate biomolecules from the eluent fluid, recover and quantify the desired biochemical.

SUMMARY OF THE INVENTION

Accordingly it is an object of the present invention to provide a column chromatography apparatus for the rapid liquid phase separation of chemicals, and in particular, biochemicals and biomolecules.

It is another object of the present invention to provide a column chromatography apparatus for ion exchange, molecular weight, adsorption/partition, hydroxylapatite, particular removal, semi-affinity and affinity chromatography separations.

It is yet another object of the present invention to provide a column chromatography apparatus for multi-stage separations in a unitary apparatus.

It is still another object of the present invention to provide a column chromatography apparatus adapted for use with a common laboratory centrifuge or ultracentrifuge.

It is still yet another object of the present invention to provide a column chromatography apparatus adapted for use with a syringe so that syringe-injected air may be utilized to create a fluid flow force.

It is a further object of the present invention to provide a column chromatography apparatus having a vented and non-vented cap for sealing the column during separation or sealing the eluate after collection.

It is another further object of the present invention to provide a column chromatography apparatus having an eluate collection portion adapted for use with laboratory analytical devices.

The aforementioned objects are accomplished, according to the present invention, by providing a column chromatography apparatus having a multi-stage separation column portion, an eluate collection portion, an adapter portion, a vented cap portion and a non-vented cap portion.

The foregoing objects, features and advantages of this invention will be apparent from the following, more particular, description of the preferred embodiments of this invention, as illustrated in the accompanying drawings, wherein like features are identified by like numerals.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a side elevational cross-sectional view of a column chromatography apparatus according to the present invention illustrating the multi-stage separation column portion.

FIG. 1*a* is a perspective exploded view of the miniaturized column chromatography apparatus according to the present invention.

FIG. 2 is a side elevational cross-sectional view of a column chromatography apparatus according to the present invention illustrating the eluate collection portion thereof.

FIG. 3 is a side elevational cross-sectional view of a column chromatography apparatus according to the

present invention illustrating the adapter portion thereof.

FIG. 4 is a side elevational cross-sectional view of a cap portion of a column chromatography apparatus according to the present invention.

FIG. 5 is a side elevational cross-sectional view of a cap portion communicating with an adapter portion of a column chromatography apparatus according to the present invention.

FIG. 6 is a side elevational cross-sectional view of a cap portion communicating with a multi-stage separation column portion of the miniaturized column chromatography apparatus according to the present invention.

FIG. 7 is a side elevational view of an adapter portion communicating with a multi-stage separation column portion of the inventive miniaturized column chromatography apparatus.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Turning to the accompanying Figures, in which like features are identified by like numerals, the preferred embodiment of a column chromatography apparatus **10** is illustrated. As illustrated by FIG. **1**, column chromatography apparatus **10** is ideally suited for laboratory and clinical use for rapid separations of organic, inorganic and biochemicals, as well as biomolecules by liquid phase chromatography. Liquid phase chromatography entails the introduction of a fluid, e.g., blood plasma or urine, into a column packed with a suitable separatory medium, the selection of which depends upon the chemical sought to be separated. After allowing the eluent to course through the length of the column, the eluate is collected in a suitable receptacle. Typically, the scientist, technician or clinician will select a separatory medium for separating the desired biochemical or biomolecule from the eluent. To collect the desired biochemical or biomolecule, the column is washed with a suitable wash, typically an aqueous acid or base, and this wash is collected and assayed. Repeated washings may recover additional quantities of the desired biochemical or biomolecule.

Column chromatography apparatus **10** consists of a generally tubular column member **12** which has an eluant inlet opening **15** and an elutate outlet opening **21**. According to the present invention eluent inlet opening **15** has a diameter which is relatively greater than the diameter of eluate outlet opening **21**. Since many biochemical assays must be performed at cold temperatures, the intermediates or products of these assays are frequently stored at extremely cold temperatures (as low as −100° C.), to avoid denaturation of proteinaceous biomolecules, the material of choice for tubular column member **12** must be thermally stable across a wide temperature range. In addition, the material must be stable in the presence of a diversity of chemicals, particularly aqueous acids or bases and be capable of withstanding the forces of sterilization by gamma and beta irradiation and gaseous ethylene oxide. It is preferable, therefore, that tubular column member **12** be made of a material, such as an ethylene-polypropylene plastic, which is sufficiently chemically and thermally inert to the above. It is further preferable to employ a plastic which is inexpensive and, thereby, disposable. According to the preferred embodiment of the present invention, tubular column member **12** is constructed of a unitary piece of milled and bored, or injection molded plastic such as that marketed by Eastman Chemical Products under the trademark TEMITE. It has been found that TENITE No. 7583-353A is particularly well suited for use with the present invention.

Tubular column member **12** tapers along its lengthwise axis, gradually tapering from eluent input opening **15** to eluate outlet opening **21**. A filter **22** is horizontally disposed adjacent to and subtending the eluent outlet **21**. Filter **22** preferably consists of a microporous polymeric plastic filter. It has been found that filters made of polyethylene, polypropylene, polyether-polyurethane, polyester-polyurethane, polyolefin, polyvinylidene flouride, ethylene-vinyl acetate, styrene-acrylonitrile or polytetrafluoroethylene are particularly well-suited to use in the present invention. Examples of such polymeric plastics include those marketed under the trademark INTERFLO by Chromex Corporation of New York, VYON, PERMAIAR, PORELLE by Pore Technology, Ltd. of Somerville, Mass. or POREX by Porex Technologies, Corp. of Fairburn, Ga. Each of these polymeric plastics are sufficiently chemically and thermally inert to render them particularly well suited for use in the inventive miniaturized column chromatography apparatus.

It is preferable, according to the preferred embodiment of the present invention, to provide a tubular column member **12** having a plurality of chambers **13**, **17** and **19** defined by successive constrictions **16** and **18** in the diameter of tubular column member **12**. Such a multi-chambered tubular column member, the technician may be packed with a single separatory medium or packed as a multi-stage separatory column. Where a single separation is required, the entire column length, or any portion thereof, may be packed with a suitable separatory medium. Where multiple separations are required, however, a single column may be differentially packed with different separatory media to achieve multiple separations. It will be recognized by those skilled in the art, that the feature of a plurality of increasingly larger diameter chambers increases the stack height of, and therefore the efficiency of, the separatory material or materials. As an example, 100 mg of a separatory medium is more efficient in a ¼-inch diameter column than in a one-inch column. Increased stack height of the separatory medium can provide a proportional increase in surface area for the eluent to contact. Accordingly, it is advantageous to provide, as in the present invention, a column which optimizes the efficiency of the separatory medium by increasing its stack height through a plurality of increasingly larger diameter chambers.

By providing a tubular column member **12** having a plurality of chambers **13**, **17** and **19**, it is possible to separate chambers **13**, **17** and **19** by disposing polymeric filters **22** therebetween at constrictions **16** and **18** respectively or at any other point along the taper of each chamber. Separation of the column into a plurality of contiguous, but differential chambers permits the successive separation of different biochemical or biomolecules with different separatory media.

It will be understood, by those skilled in the art, that any suitable separatory media and associated separatory method may be employed. In particular, the present miniaturized column chromatography apparatus is particularly well suited to rapid ion exchange, molecular weight, adsorption/partition, hydroxylapatite, particulate removal, semi-affinity and affinity chromatography separations. Accordingly, suitable separatory media

include, but are not limited to, ion exchange resin, ion exchange cellulose, ion exchange gel, sephadex, silica gel, alumina, hydroxylapatite gel, phenylborate cellulose and affinity gel. Thus, it can be readily acknowledged by those skilled in the art, that the present invention has a broad utility across the wide range of analytical and clinical chromatographic separations.

To increase the broad utility of the present invention, it has been found desirable to provide an entire miniaturized column chromatography apparatus consisting of a tubular column member **12**, a receptacle container **40**, a cap member **50** and an adapter **30**. As illustrated by FIG. **2**, receptacle container **40** consists of an elongated tapering tubular member **42** having a tapering frustoconical shaped lower portion **44**. An upper rim portion **46** of receptacle container **40** has an inner diameter which is configured to securely couple the outside diameter of a surface of tubular column member **12**. By providing corresponding inner and outer diameters of tubular column member **12** and receptacle container respectively, the technician, scientist or clinician will be able to fit the miniaturized column chromatography apparatus **10** in a standard test tube rack and standard laboratory centrifuges and ultracentrifuges without danger of adversely affecting the column or separation.

With reference to FIGS. **3**, **4** and **5**, there is shown the adapter and cap of the present invention. Cap **50** and adapter **30** are configured for multiple uses as either vented or non-vented coverings for tubular column member **12** along, tubular column member in combination with receptacle container **40** in FIG. **2** or receptacle container **40** in FIG. **2** alone. Ideally, cap **50** consists of a generally cylindrical hollow lower stalk portion **54** and a generally planar upper cap portion **51** integrally extensible from upper cap portion **51** and disposed thereupon. Upper cap portion **51** further has a lower ring portion **52** depending from the outer periphery of upper cap portion **51**. It is especially desirable, according to the contemplated preferred embodiment of the present invention, to configure hollow lower stalk portion **54** so that its outside diameter will engage within the inside diameter of an upper rim portion **14** of generally tubular column member **12**. Hollow lower stalk portion **54** is configured so that its outer diameter directly corresponds to the inner diameter of the upper rim portion **46** of receptacle container **40** in FIG. **2**, thereby creating a non-vented closure for receptacle container **40**. Further, as depicted in FIG. **6**, cap **50** is ideally configured so that the inside diameter of lower ring portion **52** corresponds to the outside diameter of the upper rim portion **14** of tubular column member **12**, thereby providing a vented closure for tubular column member **12**.

It is necessary, however, that cap **50** provide some means for venting air into the column to counteract the negative pressure created by the fluid flow through the column. Accordingly, it has been found preferable to provide venting means **53** on cap **50**. The best mode for providing venting means **53**, as contemplated according to the present invention, is to provide a plurality of venting protrusions **53** extending radially across the inner surface of upper cap portion **51** and lower ring portion **52**. The provision of venting protrusions **52** permits the free flow of eluent fluid through the column without the creation of negative pressure due to its flow through the column. Moreover, venting protrusions **52** permit a vented seal to be made between cap portion **50** and tubular column member **12** while minimizing the possibility of contamination during handling.

Adapter **30** is provided to allow usage of tubular column member **12** with syringes for injecting air into tubular column member **12**. Adapter **30** consists of generally cylindrical hollow upper portion **34**, a generally cylindrical hollow lower portion **32** depending therefrom and a radial flange **36** extending outwardly at the junction of upper portion **34** and lower portion **32**. As shown in FIG. **5**, hollow upper portion **34** is configured to have an outside diameter which corresponds to the inner diameter of the lower stalk portion **54** of cap **50**. It is also desirable, according to the preferred embodiment of the present invention to configure hollow upper portion **34** of adapter **30** so that its inner diameter corresponds to the universal luer taper standard for syringes and other medical luers as set forth by the American National Standards Institute in "Glass and Metal Luer Tapers for Medical Applications." An adapter **30** having a standardized hollow upper portion **34** is capable of being used with most standard syringe luers. Hollow lower portion **32** is configured to have an outside diameter which corresponds to the inside diameter of the upper portion **14** of tubular column member **12**.

It has been found that by employing the present miniaturized column chromatography apparatus in conventional biochemical assays, an unexpected improvement in assay accuracy has been demonstrated. For example, a present method of assaying catecholamines in plasma, urine and cerebrospinal fluid entails introduction of the eluent into a test tube to which finely powdered alumina is added. The tube is gently agitated for several minutes and centrifuged to pelletize the alumina. Upon removal from the centrifuge, the supernatant is carefully removed by hand-pipetting and discarded. The alumina is resuspended in the tube in an acid wash, allowed to react for several minutes, and recentrifuged to pelletize the alumina. The supernatant is then removed by hand pipetting and introduced into a second tube for further steps in the procedure to quantify catecholamines. Several washes with acid and their requisite centrifugations and careful removal of the supernantant may be necessary to fully recover the catecholamine fraction. It will be recognized by those skilled in the art, that the repetition of each hand-performed operation increases the likelihood of contamination and human error.

In contradistinction to present assay methods, the present invention further provides a method of assaying biomolecules which is particularly well suited for rapid analytical or clinical column chromatography separations. In particular, it has been found that about a 12–15% improvement in recovery has been achieved by employing the present miniaturized column chromatography apparatus in the present inventive method. As examples of the present method, but not to be understood as limiting the present invention, catecholamine assays were run as follows:

EXAMPLE I

Aliquots of 10 μl of a concentrated tritiated norepinephrine (^{3}H-NE) solution, suspended in a Tris buffer with 0.1 mM of dithiothreitol (DTT, 0.154 mg/ml) as an antioxidant, were added to each of three miniaturized column chromatography apparatuses (A, B and C) according to the present invention. Each of the columns was centrifuged and the eluates collected. The columns were then washed four times each with 4 ml of a deionized water/DTT solution at 4° C. Eluates were col-

lected and radioactivity was counted in a scintillation counter. Subsequently, column A was washed three times, each with 200 ul of 0.1N HCl; the eluates were similarly collected and counted. In a like manner, columns B and C were washed three times each with 200 ul of 0.5N HCl; the eluates were collected and counted. The percent recoveries of norepinephrine are summarized by the following table:

TABLE A

Wash	Column A	Column B	Column C
Water:	2.0%	2.1%	4.9%
Acid			
0.1 N HCl	78.5%	—	—
0.5 N HCl	—	75%	81.0%

EXAMPLE II

The same procedures were followed as in Example I, above, except 10 ul of the ^{3}H-NE diluted solution was added to 0.1 mM DTT solution, 1000 pg/nl non-radioactive dopamine, epinephrine and norepinephrine (DEN) was added. The resulting solution was then introduced into three columns packed as follows:

Column A: 50 mg alumina
Column B: 100 mg alumina
Column C: 200 mg alumina

After water washes followed by three washes each with 200 ul 0.1N HCl and one wash of 200 ul 0.5N HCl the following recoveries were obtained:

TABLE B

Wash	Column A	Column B	Column C
Water:	5.2%	2.6%	0.8%
Acid:	48.33%	64.35%	63.45%

Thus, it will be readily noted, by those skilled in the art, that excellent recoveries are possible with the assay according to the present invention. In particular, as indicated by the aforementioned Examples, a catecholamine assay following the present invention is best carried out utilizing three washes of 0.5N HCl and either a 100 mg or 200 mg alumina packed column.

While the invention has been particularly shown and described in reference to the preferred embodiments thereof, it will be understood by those skilled in the art that changes in form and details may be made without departing from the spirit and scope of the invention.

I claim:

1. A miniaturized column chromatography apparatus, comprising:

a generally tubular column member having a plurality of chambers, each of said plurality of chambers being disposed in fluid flow communication with another of said plurality of chambers, said tubular column member further having an eluent inlet and eluate outlet, said inlet having a relatively larger diameter than and in fluid flow communication with said eluate outlet, said eluate outlet further having a generally frusto-conical shape;

a planar disc-shaped porous filter horizontally disposed within said generally tubular column member and subtending said eluate outlet;

chromatographic separatory medium packed within said generally tubular column member, said chromatographic separatory medium being selected dependent upon the materials to be separated;

vented capping means for closing said eluate inlet disposed within eluate inlet opening and comprising a cap and a cap adapter, wherein said cap further comprises a generally cylindrical hollow lower stalk portion and a generally planar upper cap portion having a lower ring portion depending therefrom, wherein said lower ring portion has an inside diameter corresponding to an outside diameter of an upper rim portion of said generally tubular column member; and wherein said cap adapter further comprises a generally cylindrical hollow upper portion, a generally cylindrical hollow lower portion depending therefrom and a radial flange extending outwardly from an upper surface of said generally cylindrical lower portion, said upper portion having outside diameter corresponding to an inner diameter of said generally cylindrical hollow lower stalk portion of said cap and said lower portion having an outside diameter corresponding to an inside diameter of an upper portion of said generally tubular column member; and

an eluate receiving member having a rounded bottom portion for forming a pellet therein; said eluate receiving member being disposed in fluid communication with said generally tubular column member.

2. The miniaturized column chromatography apparatus according to claim 1, wherein each of said plurality of chambers further comprising an outlet and an inlet opening thereto, wherein each of said outlet openings has a relatively smaller diameter than each of said associated inlet openings.

3. The miniaturized column chromatography apparatus according to claim 2, wherein said generally tubular column member further a unitary column member constructed of a chemically and thermally inert plastic material.

4. The miniaturized column chromatography apparatus according to claim 2, wherein said generally tubular column member further comprises a unitary column member constructed of an ethylene and propylene plastic.

5. The miniaturized column chromatography apparatus according to claim 2, wherein each of said outlet openings further comprises a porous filter subtending said outlet opening.

6. The miniaturized column chromatography apparatus according to claim 5, wherein each of said plurality of chambers further comprises different chromatographic separatory media packed therein, each of said different chromatographic separatory media being selected dependent upon the materials to be separated.

7. The miniaturized column chromatography apparatus according to claim 6, wherein said chromatographic separatory medium is selected from the group consisting of ion exchange resin, ion exchange cellulose, ion exchange gel, sephadex, silica gel, alumina, hydroxyapatite gel, phenylborate cellulose and affinity gel.

8. The miniaturized column chromatography apparatus according to claim 2, wherein said chromatographic separatory medium is selected from the group consisting of ion exchange resin, ion exchange cellulose, ion exchange gel, sephadex, silica gel, alumina, hydroxyapatite gel, phenylborate cellulose and affinity gel.

9. The miniaturized column chromatography apparatus according to claim 1, wherein said generally tubular column member further comprises a unitary column member constructed of a chemically and thermally inert plastic material.

10. The miniaturized column chromatography apparatus according to claim 1, wherein said generally tubular column member further comprises a unitary column member constructed of an ethylene and propylene plastic.

11. The miniaturized column chromatography apparatus according to claim 1, wherein said porous filter further comprises a polymeric plastic material.

12. The miniaturized column chromatography apparatus according to claim 1, wherein said porous filter further comprises a polymeric plastic material selected from the group consisting of polyethylene, polypropylene, polyether-polyurethane, polyester-polyurethane, polyolefin, polyvinylidene flouride, ethylene-vinyl acetate, styrene-acrylonitrile and polytetrafluoroethylene.

13. The miniaturized column chromatography apparatus according to claim 1, wherein said generally tubular column member further comprises a plurality of said porous filters horizontally disposed therein forming a plurality of chambers therebetween.

14. The miniaturized column chromatography apparatus according to claim 13, wherein each of said plurality of chambers further comprises different chromatographic separatory media packed therein, each of said different chromatographic separatory media being selected dependent upon the materials to be separated.

15. The miniaturized column chromatography apparatus according to claim 1, wherein each of said chambers further comprises a porous filter horizontally disposed within said chamber and subtending an outlet opening thereof.

16. The miniaturized column chromatography apparatus according to claim 15, wherein each of said plurality of chambers further comprises different chromatographic separatory media packed therein, each of said different chromatographic separatory media being selected dependent upon the materials to be separated.

17. The miniaturized column chromatography apparatus according to claim 1, wherein said chromatographic separatory medium is selected from the group consisting of ion exchange resin, ion exchange cellulose, ion exchange gel, sephadex, silica gel, alumina, hydroxyapatite gel, phenylborate cellulose and affinity gel.

18. The miniaturized column chromatography apparatus according to claim 1, wherein said multi-stage column member further comprises a plurality of said porous polymeric filters subtending an outlet opening of at least one of said chambers of said multi-stage column member.

19. The miniaturized column chromatography apparatus according to claim 18, wherein each of said plurality of chambers having an outlet opening subtended by said porous polymeric filters further comprises different chromatographic separatory media packed therein, each of said different chromatographic separatory media being selected dependent upon the materials to be separated.

20. A miniaturized column chromatography apparatus, comprising, in combination:

a multi-stage column member having an eluate inlet and eluent outlet opening and a plurality of chambers in fluid flow communication disposed therebetween, each of said chambers having an inlet and outlet opening, each of said inlet openings further having a relatively larger diameter than a communicating outlet opening, said eluate outlet further having a generally frustro-conical shape and a central bore passing therethrough;

at least one of a plurality of porous polymeric disc-shaped filters horizontally disposed within said multi-stage column member and subtending said eluate outlet and at least one of said outlet openings of said plurality of chambers;

at least one of a plurality of chromatographic separatory media packed within said generally tubular generally tubular column member, said chromatographic separatory media being selected dependent upon the materials to be separated wherein a different and distinct one of said at least one of a plurality of chromatographic separatory media is packed in each of said plurality of chambers of said multi-stage column member for separating different and distinct materials from a solution;

a cap member having a generally cylindrical hollow lower stalk portion and a generally planar upper cap portion having a lower ring portion depending therefrom, wherein said lower ring portion has an inside diameter corresponding to an outside diameter of an upper rim portion of said generally tubular column member;

a cap adapter having a generally cylindrical hollow upper portion, a generally cylindrical hollow lower portion depending therefrom and a radial flange extending outwardly from an upper surface of said generally cylindrical lower portion, said upper portion having outside diameter corresponding to an inner diameter of said generally cylindrical hollow lower stalk portion of said cap and said lower portion having an outside diameter corresponding to an inside diameter of an upper portion of said generally tubular column member; and

an eluate receiving member disposed in fluid communication with said generally tubular column member.

21. The miniaturized column chromatography apparatus according to claim 20, wherein said generally tubular column member further comprises a unitary column member constructed of a chemically and thermally inert plastic material.

22. The miniaturized column chromatography apparatus according to claim 20, wherein said porous polymeric filter further comprises a plastic material selected from the group consisting of polyethylene, polypropylene, polyether-polyurethane, polyester-polyurethane, polyolefin, polyvinylidene fluoride, ethylene-vinyl acetate, styrene-acrylonitrile and polytetrafluoroethylene.

23. The miniaturized column chromatography apparatus according to claim 20, wherein said chromatographic separatory medium is selected from the group consisting of ion exchange resin, ion exchange cellulose, ion exchange gel, sephadex, silica gel, alumina, hydroxyapatite gel, phenylborate cellulose and affinity gel.

24. The miniaturized column chromatography apparatus according to claim 20, wherein said cap portion of said capping means further comprises venting means disposed on said cap portion for alleviating negative pressure within said generally tubular column member during a separation run.

* * * * *

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United States Patent [19]

Zare et al.

[11] Patent Number: 6,136,187

[45] Date of Patent: *Oct. 24, 2000

[54] **SEPARATION COLUMN CONTAINING POROUS MATRIX AND METHOD OF PACKING COLUMN**

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[73] Assignee: **The Board of Trustees of the Leland Stanford Junior University**, Stanford, Calif.

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: **08/987,287**

[22] Filed: **Dec. 9, 1997**

[51] **Int. Cl.**[7] **B01D 15/08**

[52] **U.S. Cl.** **210/198.2**; 210/656; 96/101

[58] **Field of Search** 210/198.2, 635, 210/656, 659; 95/83, 88; 96/101

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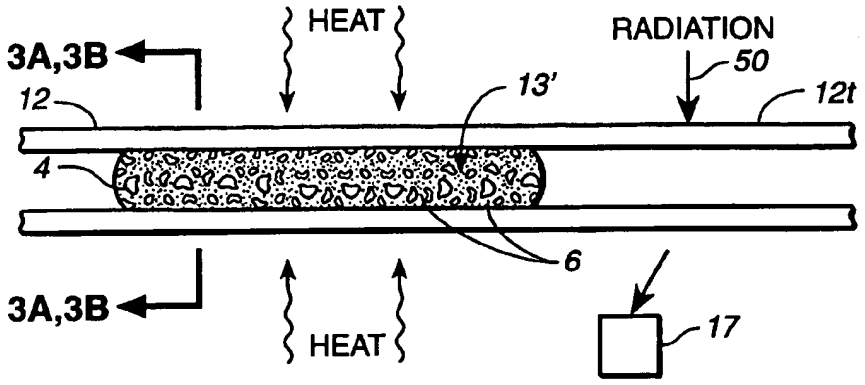
[57] **ABSTRACT**

A mixture of chromatographic particles and a solution of water, alcohol and metal alkoxide may be injected by means of a syringe into a capillary column as a gel. The volatile components in the gel are evaporated by means of heating and gas pressure reduction to form a porous sol-gel glass matrix attached to the inner wall of the separation channel. The pores are large enough for the passage of protons, neutral and ionic species but are too small to permit significant leaching of the chromatographic particles. The separation column so formed requires no frits to maintain the glass matrix in place in the column. Electrical potential difference and/or pressure difference may be applied to cause fluid flow in the separation column to cause electrophoretic and chromatographic separation.

29 Claims, 3 Drawing Sheets



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"'Laterally aggregated'polyacrylamide gels for electrophoresis," P.G. Righetti et al., *Electrophoresis*, No. 13, Sep./Oct. 1992, pp. 587–595.

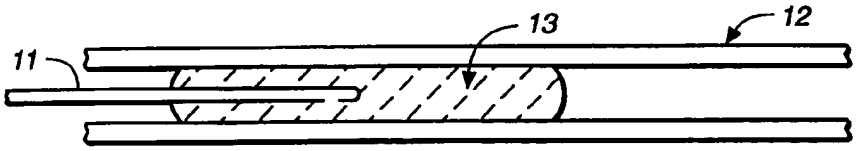


FIG._1

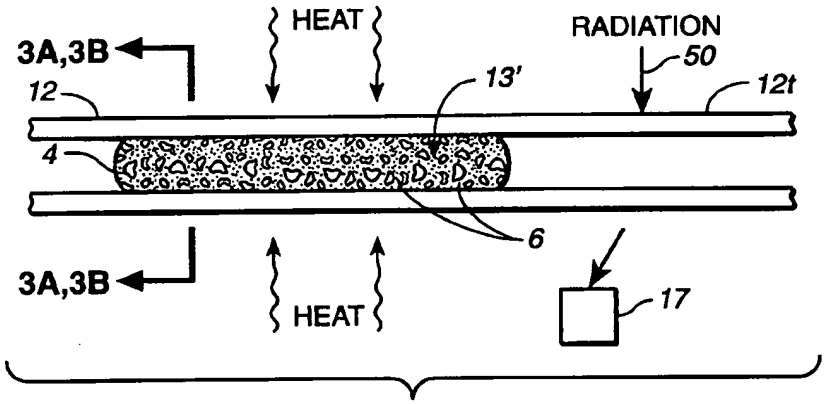


FIG._2

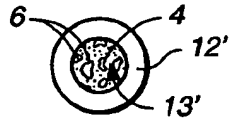


FIG._3A

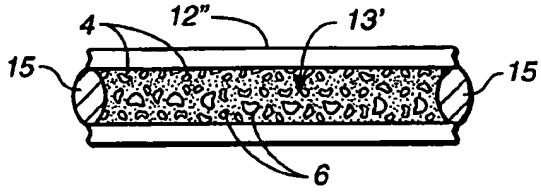


FIG._3B

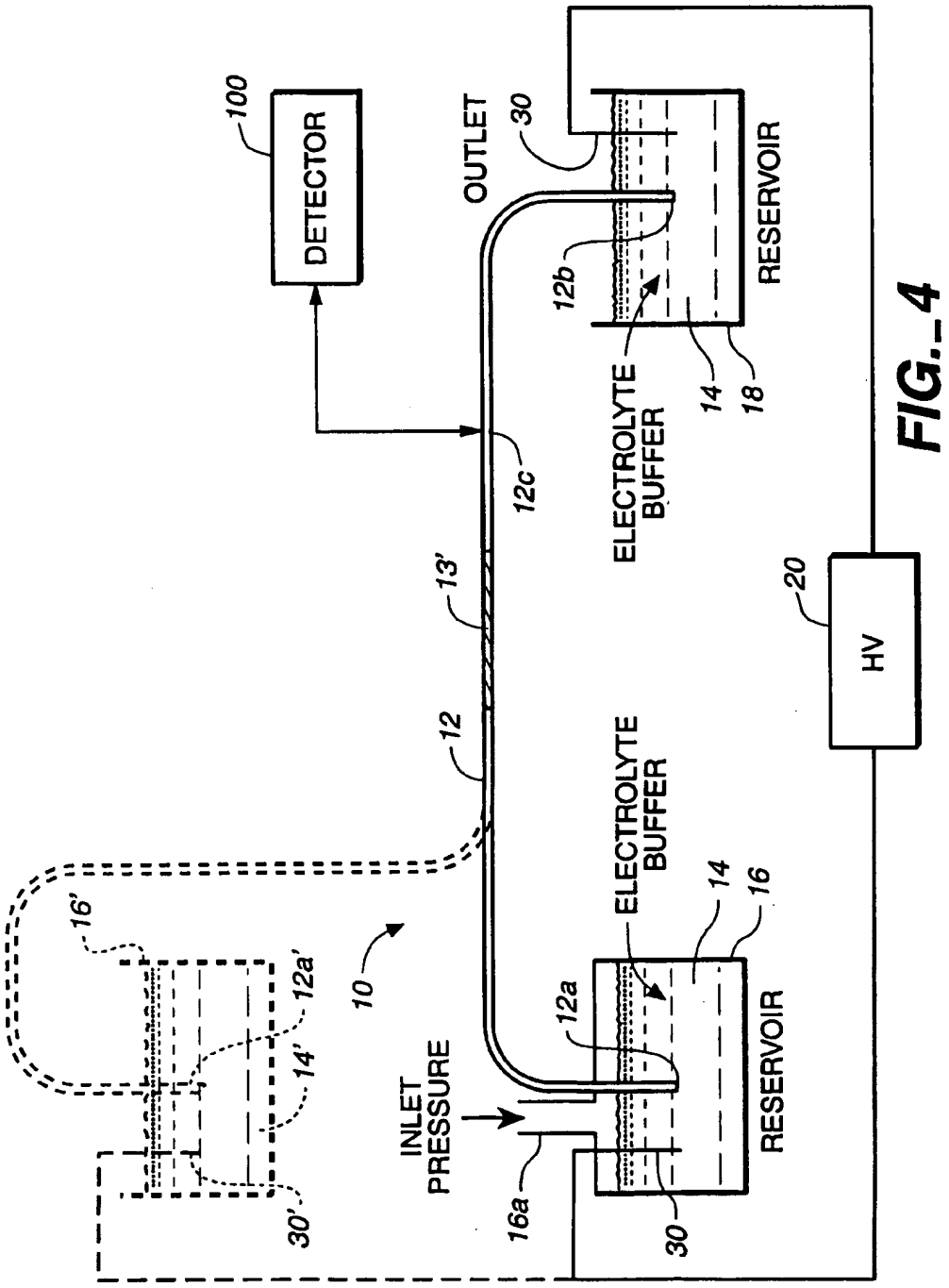

100
DETECTOR
OUTLET
30
RESERVOIR
12b
ELECTROLYTE
BUFFER
14
18
12c
13'
20
HV
12
ELECTROLYTE
BUFFER
14
16
16'
12a'
10
12a
14'
INLET
PRESSURE
16a
30'
30

FIG._4

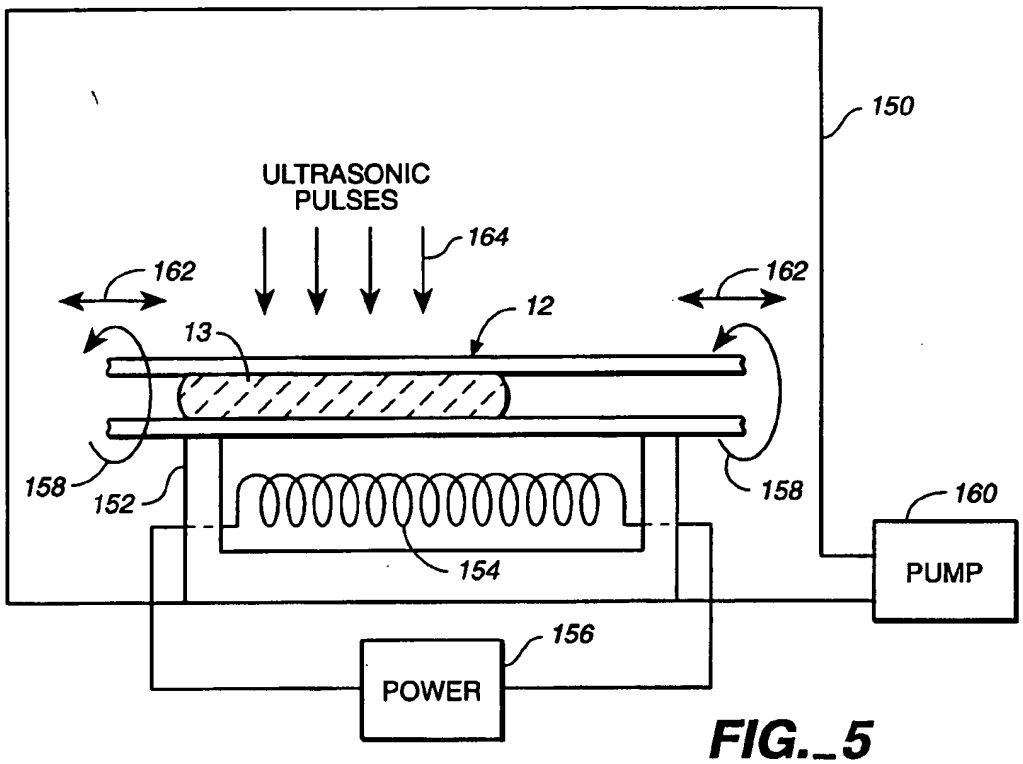

150
ULTRASONIC
PULSES
164
162
162
12
13
158
152
158
160
154
PUMP
156
POWER

FIG._5

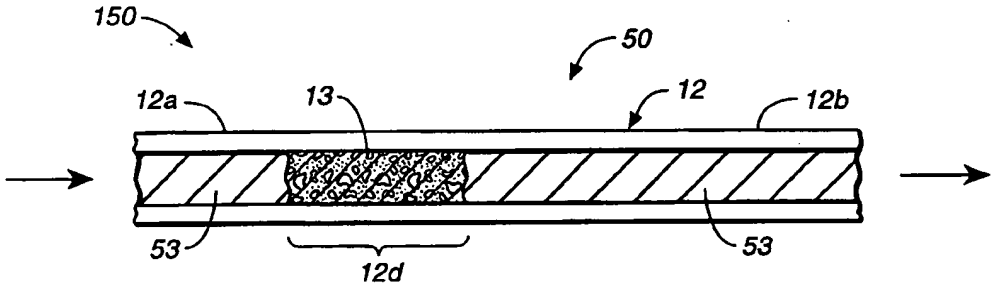

150
50
12a
13
12
12b
53
53
12d

FIG._6

SEPARATION COLUMN CONTAINING POROUS MATRIX AND METHOD OF PACKING COLUMN

BACKGROUND OF THE INVENTION

The invention relates to a separation system employing a column containing a porous matrix embedded with chromatographic particles and a method of packing a channel with the matrix to make the column.

Capillary zone electrophoresis (CZE), with its high peak capacity (i.e., the number of peaks separated per unit time), has long been proven to be an attractive analytical technique for separating ionic species by their electrophoretic mobilities. The separation of neutral species via CZE, however, has remained more problematic. To improve the separation of neutral species via capillary electrophoresis, the technique of capillary electrochromatography (CEC) has been employed, which is a promising technique that seeks to combine the advantages of capillary electrophoresis and chromatography as described in the article by M. T. Dulay et al. in *Chromatogr. A.*, 725 (1996) pp. 361–365).

In CEC, the separation of uncharged analytes is based on partitioning of chromatographic particles such as octadecylsilica, while the separations of charged analytes are based on both partitioning and electrophoretic mobility. Existing techniques for the preparation of packed capillary columns are based on either a slurry packing method or an electrokinetic packing method of small-bore capillary columns. The electrokinetic packing method may be more advantageous than a slurry packing method for the preparation of packed capillary columns with micron-sized inner diameters. Disadvantages of the electrokinetic packing method include the limited choices of chromatographic phases (i.e., only charged particles can be used) and the need for both inlet and outlet frits to prevent the chromatographic particles from leaving the capillary column. This causes the columns to be difficult and time consuming to make.

It is therefore desirable to provide a separation column with improved characteristics and that are easy to make.

SUMMARY OF THE INVENTION

One aspect of the invention is directed towards a separation column comprising a separation channel having a channel wall and a separation medium in the channel. The medium includes a porous matrix attached to the channel wall and chromatographic particles embedded in the matrix forming a packed channel. The channel has no frit therein adjacent to the separation medium.

Another aspect of the invention is directed towards an apparatus for separating a sample into its components, comprising a separation channel having a channel wall; a separation medium in the channel, and means for causing a fluid containing a sample present in a channel to flow and the sample to separate. The medium includes a porous matrix attached to the channel wall and chromatographic particles embedded in the matrix, forming a packed channel. The channel has no frit therein adjacent to the separation medium.

One more aspect of the invention is directed towards a method for making a separation column, comprising introducing a mixture of chromatographic particles and a solution of a monomer and a cross-linking reagent into a separation channel, such channel having a wall; and causing the mixture to form a porous matrix attached to the channel wall with said particles embedded therein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a partially cross-sectional and partially schematic view of a section of a separation column along a longitudinal axis of the column and of a needle portion of a syringe for injecting a mixture of chromatographic particles and a solution of water, alcohol and a metal alkoxide into the column to illustrate a preferred embodiment of the invention.

FIG. **2** is a cross-sectional view of a portion of the column of FIG. **1** along a longitudinal axis of the column, where the volatile components in the mixture injected into the column have been removed to form a porous sol-gel with chromatographic particles embedded therein to illustrate the invention.

FIGS. **3A** and **3B** are cross-sectional views of the separation column shown in FIG. **2** along the line **3A, 3B–3A, 3B** in FIG. **2** to illustrate two different embodiments of the separation column.

FIG. **4** is a partially schematic and partially cross-sectional view of a separation system employing the separation column of FIG. **2** to illustrate a preferred embodiment of the invention.

FIG. **5** is a schematic view of a system for making the separation column of FIG. **2**.

FIG. **6** is a cross-sectional view of a portion of a separation column along a longitudinal axis of the column to illustrate another embodiment of the column and an alternative method for packing the column.

For simplicity in description, identical components are labeled by the same numerals in this application.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the preferred embodiment, this invention employs a metal alkoxide sol-gel process. The metal alkoxide sol-gel process is a method of preparing metal oxide glasses by hydrolyzing a solution of water, alcohol, and a metal alkoxide source. The sources of these metal oxides, or silanes, are the alkoxy compounds of type $R_nSi(OR')_{4-n}$ as described by C. J. Brinker et al. in *Sol-Gel Science*, Academic Press, Inc., New York, N.Y., 1990. The most commonly used of these compounds is tetraethylorthosilicate (TEOS, $Si(OC_2H_5)_4$), although other compounds such as titanates, and zirconates may also be used for this invention. As these substances polymerize, gelation of the solution occurs. If the volatile solvents in the wet gel are allowed to evaporate, the gel shrinks and hardens, creating a hard porous glass.

Since sol-gel glasses are formed from solution, other molecules can be embedded inside the pores or inside the cavities created. When the solvent evaporates from the gel, the glass that is created is porous. The porosity of the glass allows for the diffusion of protons (and other neutral or ionic species) through the channels. These pores, however, must be large enough to allow species diffusion, but small enough that significant amounts of chromatographic materials cannot leave the xerogel matrix.

FIG. **1** is a partially cross-sectional and partially schematic view of a portion of a separation column **12** along a longitudinal axis of the column and a needle portion **11** of a syringe (not shown) injecting a mixture **13** of chromatographic particles and a solution to illustrate the preferred embodiment of the invention. As shown in FIG. **1**, a needle portion **11** is used to inject into a channel within a tube **12**, a mixture **13** of chromatographic particles in a solution of water, alcohol and a metal alkoxide. This mixture forms a

wet gel **13** in tube **12**. After the volatile solvents in the wet gel **13** have evaporated, the gel **13** shrinks and hardens, creating a hard porous glass **13'** shown in FIG. **2**.

FIG. **2** is a cross-sectional view of column **12** along a longitudinal axis of the column and the hard porous glass **13'** in the column resulting from evaporation of the volatile solvents in the wet gel **13** of FIG. **1**. As shown in FIG. **2**, glass **13'** includes pores **4** and chromatographic particles **6** embedded therein. As noted above, the porosity of the glass **13'** allows for the diffusion of protons and other neutral or ionic species, but small enough that significant amounts of chromatographic particles **6** cannot leave the glass matrix **13'**. Glass **13'** forms a secure bond to the inner wall of the tube **12**, so that no frit is required adjacent to the glass **13'** to maintain the matrix glass **13'** in place in tube **12**. While pores **4** are big enough to allow diffusion of protons, neutral and ionic species, they are too small for most of the chromatographic particles **6**. This prevents leaching of the particles when a fluid is passed through the glass matrix. Thus, when a sample is carried by fluid through glass **13'**, the sample components will interact with the chromatographic particles **6** and become partitioned or separated.

Tube **12** can have many different cross-sections, such as a circular cross-section shown in FIG. **3A**, the cross-section being normal to a longitudinal axis of the tube, shown as tube **12'**. Alternatively, tube **12** can have an elongated cross-section as shown in FIG. **3B**, where the tube is formed by two flat plates **12"** where the tube is formed by sealing the adjacent edges of the two plates by means of an adhesive **15**. Such and other cross-sections are possible for tube **12** and are within the scope of the invention. In some embodiments, the internal dimension of tube or channel **12** may range from about 5 to about 3,000 microns. Where tube **12** is a capillary, its internal dimension may range from about 5 to about 300 microns.

Chromatographic particles **6** may comprise uniformly sized particles of the same type, or a mixture of different types of particles of different sizes. Preferably, the particles are of dimensions greater than 0.2 microns.

FIG. **4** is a partially schematic and partially cross-sectional view of a separation system employing a column **12** to illustrate the preferred embodiment of the invention. As shown in FIG. **4**, the inlet end **12*a*** of the column is immersed in an electrolyte buffer **14** contained in a reservoir **16**. The outlet end **12*b*** is immersed in an electrolyte buffer in reservoir **18**. An electrical potential of several kilovolts is applied by high voltage source **20** through electrodes **30** between electrolyte buffer **14** in input and output reservoirs **16, 18**, causing a potential difference and electric field along the column **12**. Such potential difference causes an electroosmotic flow in tube **12** from reservoir **16** towards reservoir **18**. A sample may be introduced into inlet **12*a*** by means known to those skilled in the art, such as by gravity or by electrokinetic injection. Such sample would be caused to separate in tube **12** due to electrophoresis. In addition, the interaction between the sample components (which may be uncharged or neutral electrically) and the chromatographic particles **6** in the glass matrix **13'** causes chromatographic separation of the components as well.

Instead of, or in addition to, the use of electrical potential to cause fluid flow in column **12**, the fluid flow can also be caused by applying a pressure differential along the column **12**, which, in addition to or instead of the electric field applied along the column, causes fluid flow from reservoir **16** to reservoir **18**. This can be performed by using an enclosed reservoir **16** except for an inlet **16*a***, through which gas pressure is applied as shown in FIG. **4**. The gas pressure may be supplied by means of a pump (not shown) for example. The gas pressure applied through inlet **16*a*** causes buffer **14** to be pushed downward and the buffer to flow into the inlet **12*a*** and column **12** to reservoir **18**. Instead of using a pump to apply a pressure differential in column **12**, a pressure differential may also be applied by raising the column inlet **12*a*** to a position **12*a'*** at an elevation higher than the outlet end **12*b*** of the column, as shown in dotted lines in FIG. **4**. Thus, as shown in dotted lines, the new positions of the inlet **12*a'*** of the column, of reservoir **16'** containing buffer **14'** and electrode **30'** immersed in the buffer in reservoir **16'** are all at elevations above the outlet end **12*b*** of the column. Alternatively, instead of raising the inlet end **12*a***, the same goal can be achieved by lowering outlet end **12*b*** of the tube, by lowering reservoir **18**, electrode **30** in such reservoir and end **12*b***. Or, the pressure in reservoir **18** can be reduced by means of a pump (not shown) to one below that of reservoir **16** to achieve a pressure differential between ends **12*a***, **12*b***, and to create fluid flow in tube **12**.

In the embodiment of FIG. **2**, only a portion of the column **12** is filled with the porous glass with chromatographic particles embedded therein, and the remaining portion of the column is not filled with the porous glass. In an arrangement similar to that in prior detection schemes, the separated sample components may be detected optically, such as by laser induced fluorescence as described in U.S. Pat. No. 4,675,300 in a transparent column portion **12*t*** that is transparent to radiation and downstream from glass **13'** as shown in FIGS. **2** and **4**. As shown in FIG. **2**, the portion **12*t*** does not contain any of the chromatographic particles present in glass matrix **13'** and transmits radiation without significant scattering, such as UV or visible light. The sample components passing through such portion **12*t*** may be illuminated by means of radiation along arrow **50** to induce fluorescence, where the induced fluorescence may be detected by means of a detector **17** preferably placed away from the path of radiation **50**. This is shown schematically in FIG. **4**, where a detector **100** detects the separated sample components at location **12*c***. When detector **100** detects the sample components by means of laser induced fluorescence, the sample components may need to be first tagged by means of a fluorophore and detector **100** includes a laser source as well as a photodetector.

In order to facilitate the evaporation of the volatile solvents in mixture **13** in FIG. **1**, the tube **12** containing mixture **13** may be placed in an oven chamber **150** as shown in FIG. **5**. Tube **12** is supported by a pedestal **152** in the oven chamber and heat is applied by a heating coil **154** through which a current is passed by means of a power supply **156**. To further facilitate the evaporation of volatile solvents, the gas pressure in oven chamber **150** is reduced by means of a pump **160**. While both heating and reduction of gas pressure may be used together to accelerate the evaporation of volatile solvents, heating may be used without pressure reduction and vice versa; all such variations are within the scope of the invention. When the volatile solvents have evaporated, mixture **13** then forms a porous sol-gel glass that is attached securely to the inner walls of tube **12**, with the chromatographic particles **6** embedded therein. It has been found that in some instances, a hardened porous sol-gel glass may form in about 24 hours.

To achieve a more uniform distribution of the chromatographic particles in the sol-gel, it may be desirable to agitate the mixture **13** when the volatile components are escaping from the mixture. This can be done for example by rotating

the tube along arrows **158**, shaking the tube along arrows **162**, or supplying ultrasonic pulses along arrows **164** from an ultrasonic source (not shown) as shown in FIG. **5** in a manner known to those skilled in the art.

With this monolithic packing method, chromatographic materials that are charged and uncharged in nature can be embedded into the sol-gel matrix. Different functionalized/derivatized sol-gel precursors can be used to prepare sol-gel glasses with different physical properties, such as pore size and surface charge. The pore size may be selected by choosing an appropriate sol-gel precursor. For example, to obtain larger pores, tetramethylorthosilicate may be used as the precursor instead of tetraethylorthosilicate indicated above.

Mixture **13** may be prepared as follows. Micron or submicron sized chromatographic particles, such as octadecylsilica may be added to a solution of water, alcohol, acid (optional) or base (optional) and a metal oxide source. The metal oxide source may include a silicate, titanate or zirconate. The resulting solution or mixture **13** formed is injected by syringe into a column and heated overnight. No pre-fabricated frits is required.

Instead of using a syringe to inject the mixture **13** into a channel as described above in reference to FIG. **1**, the mixture **13** may be introduced by means of a pressure differential as illustrated in FIG. **6**, which is a cross-sectional view of a tube **12** along a longitudinal axis. As shown in FIG. **6**, a pressure differential is applied between the inlet end **12***a* and outlet end **12***b*, such as by means of a pump (not shown), in order to introduce a mixture **53** of water, alcohol, acid (optional) or base (optional) and a metal oxide source, without chromatographic particles embedded therein, followed by a mixture **13** of chromatographic particles with a solution such as solution **53**, which is again followed by solution **53** to fill tube **12**. Solution **53** and mixture **13** are then treated in the manner described above in reference to FIG. **5** to remove the volatile components and to form a porous sol-gel throughout the column **12**. Only the section **12***d* of the tube containing the porous sol-gel glass with chromatographic particles embedded therein is used for chromatographic separation. The remaining sections of the tube next to section **12***d* contain a porous sol-gel glass containing no chromatographic particles and, therefore, do not scatter light used for detection, so that the scheme illustrated in FIG. **4** above may be used for detecting separated sample components in a similar manner employing the column of FIG. **6** that has been filled by a porous glass. The column **150** of FIG. **6** is advantageous in that the entire tube is filled by the porous sol-gel glass so that there will no significant pressure differential between the fluid in the porous glass in section **12***d* and that in the porous glass in the remaining sections of the tube. This enhances separation performance.

The pressure differential between the inlet and outlet ends **12***a*, **12***b* may be created in many ways, such as by pushing (i.e. by increasing pressure) the mixtures **53**, **13** into the inlet end **12***a*, or by reducing the gas pressure at outlet end **12***b* to draw in solution **53** and mixture **13** as described (since such solution and mixture are under the higher atmospheric pressure at end **12***a*), by means of a pump (not shown). The inlet end **12***a* may also be placed at a higher elevation compared to outlet end **12***b* so that solution **53** and mixture **13** may be introduced by hydrostatic pressure differential.

The packed "fritless" column columns **12** will facilitate the analysis of complex mixtures that may contain charged and/or uncharged compounds. The separation of a mixture of uncharged organic compounds has been demonstrated using a column packed in this manner. Advantages of the disclosed method include (i) easy and rapid injection of the hydrolysis reaction solution into the column, (ii) the elimination of inlet and outlet frit fabrication, (iii) incorporation of charged or uncharged chromatographic materials in the sol-gel matrix, (iv) UV transparency of the sol-gel glass, (v) potential for automation of many samples, and (vi) potential for large-scale preparative use. This results in a total column preparation time of approximately 24 hours and avoids the use of high pressures for post-column conditioning. The use of high voltages is also avoided during column preparation. This system is superior to both the electrokinetic and slurry packing methods.

Instead of using a sol-gel process as described above, other types of polymerization processes may be used, such as that described in the article "Macroporous Polyacrylamide/Poly(ethylene glycol) Matrixes as Stationary Phrases in Capillary Electrochromatography," by Anders Palm and Milos V. Novotny, *Analytical Chemistry*, Vol. 69, No. 22, Nov. 15, 1997, pp. 4499–4507; or the articles by P. G. Righetti, B. C. W. Brost and R. S. Snyder, in *J. Biochem. Biophys. Methods*, 1981, No. 4, pp. 347–363, and by P. G. Righetti, S. Caglio, S. Saracchi and M. Quaroni in *Electrophoresis*, 1992, No. 13, pp. 587–595. As described in these three articles, a porous matrix may be formed by polymerizing a solution of a monomer and a cross-linking reagent or initiator. If said solution is mixed with chromatographic particles and such mixture is used instead of mixture **13** to form the porous matrix with chromatographic particles embedded therein, such matrix may also be used in a separation column for separating a sample into its components in the manner described above. When such a mixture is polymerized, the matrix forms a secure bond to the inner wall of the separation channel so that no frit is necessary to keep the matrix in place. The pores formed are big enough to permit diffusion species but small enough to prevent significant leaching of the chromatographic particles trapped therein. The porous matrix without the particles is transparent to radiation so that the configuration of FIG. **6** described above may be used, where the entire tube is filled with the porous matrix but only a section of the matrix is embedded with chromatographic particles. In this manner, the separated components may be detected downstream from the particles by a detector in a known manner, such as by means of laser induced fluorescence detection. The above-described method for introducing the mixture **13** may also be used for introducing a mixture of the particles with other types of monomers and cross-linking reagents (optional), such as acrylamide or ethylene glycol and an optional base or acid acting as a crosslinking reagnet.

A porous matrix (whether or not embedded with particles) may be formed by heating or supplying radiation to a solution of a monomer such as acrylamide or ethyleneglycol and a cross-linking reagent (optional) such as a base or acid to form a macroporous polyacrylamide or poly(ethylene glycol) matrix. The polymerization is achieved thermally or by photochemistry. In reference to the article by Palm and Novotny, since chromatographic particles are used in this invention for sample separation, there is no need in this invention to include alkyl ligands as described in the Palm and Novotny article. Polymerization techniques different from the above may also be used for forming the porous matrix; such and other variations are within the scope of the invention.

While the invention has been described above by reference to various embodiments, it will be understood that

different changes and modifications may be made without departing from the scope of the invention, which is to be defined only by the appended claims and the equivalents thereof.

What is claimed is:

1. An separation column comprising:

a capillary separation channel having a channel wall; and

a separation medium in the channel, said medium including a porous matrix attached to the channel wall and micron or submicron sized chromatographic particles embedded in the matrix forming a packed channel, said channel having no frit therein, said matrix including a glass.

2. The column of claim 1, said column having an IC internal dimension in the range of between 5 and 5,000 microns.

3. The column of claim 1, said channel having an elongated cross-section.

4. The column of claim 1, said column having a first portion that is filled with said separation medium and a second portion adjacent to said first portion that transmits radiation.

5. The column of claim 4, wherein said second portion does not contain said separation medium.

6. The column of claim 1, wherein said matrix has pores therein large enough for passage of neutral and charged species but too small for passage of the chromatographic particles.

7. The column of claim 1, said particles being larger than 0.2 micron in dimensions.

8. The column of claim 1, said particles being a mixture of different types of particles of different sizes.

9. The column of claim 1, wherein said glass includes a silicate, titanate or zirconate.

10. The column of claim 1, said column being filled with a porous matrix, said matrix having embedded therein chromatographic particles in only a section of the column.

11. An apparatus for separating a sample into its components, comprising:

a capillary separation channel having a channel wall;

a separation medium in the channel, said medium including a porous matrix attached to the channel wall and micron or submicron sized chromatographic particles embedded in the matrix, forming a packed channel, said channel having no frit therein, said matrix including a glass; and

a device causing a fluid containing a sample present in the channel to flow and the sample to separate.

12. The apparatus of claim 11, said channel being a capillary having an internal dimension in the range of between 5 and 300 microns.

13. The apparatus of claim 11, said channel having a first section that is filled with said separation medium and a second section adjacent to said first portion that transmits radiation.

14. The apparatus of claim 13, wherein said second section does not contain said separation medium.

15. The apparatus of claim 13, said second section containing a porous matrix not embedded with chromatographic particles.

16. The apparatus of claim 15, said channel filled with a porous matrix.

17. The apparatus of claim 13, said device causing the sample to pass through the second section, said apparatus further comprising:

means for applying radiation to the sample in the second section; and

means for detecting radiation from the second section to detect components of the sample passing through the second section.

18. The apparatus of claim 11, said causing means applying an electric field along the channel, so that the sample separates by electrophoresis and chromatography.

19. The apparatus of claim 11, said device applying a pressure differential along the channel, so that the sample separates by chromatography.

20. The apparatus of claim 11, said causing means applying both an electric field and a pressure differential along the channel, so that the sample separates by electrophoresis and chromatography.

21. The apparatus of claim 11, wherein said matrix has pores therein large enough for passage of neutral and charged species but too small for passage of the chromatographic particles.

22. The apparatus of claim 11, said particles being larger than 0.2 micron in dimensions.

23. The apparatus of claim 11, wherein said glass includes a silicate, titanate or zirconate.

24. The apparatus of claim 11, said column being filled with a porous matrix, said matrix having embedded therein chromatographic particles in only a section of the column.

25. A separation column comprising:

a capillary separation channel having a channel wall; and

a separation medium in the channel, said medium including a porous matrix attached to the channel wall and micron or submicron sized chromatographic particles embedded in the matrix forming a packed channel, said matrix including an a glass material.

26. A separation column, useful for separating different species in a sample, comprising:

a capillary separation channel having a channel wall; and

a glassy, porous matrix attached to the channel wall, the matrix having micron or submicron sized chromatographic particles embedded in the matrix, the particles adapted to interact with species in the sample and to cause separation of sample species when passed through the matrix.

27. The column as in claim 26 wherein the channel is a capillary having an internal dimension defined by a capillary wall and being from about 5 to about 300 microns.

28. The column as in claim 27 wherein the matrix entirely fills the capillary in at least a first section thereof.

29. The column as in claim 28 wherein attachments between capillary wall, matrix and the particles embedded therein are sufficient to maintain the matrix and embedded particles within the capillary without a frit.

* * * * *

Nov. 18, 1969 R. F. HORNBECK 3,478,886

GRAFT-COPOLYMER COLUMN SUPPORT MATERIAL FOR LIQUID-LIQUID PARTITION CHROMATOGRAPHY

Filed March 31, 1967

Fig. 1.

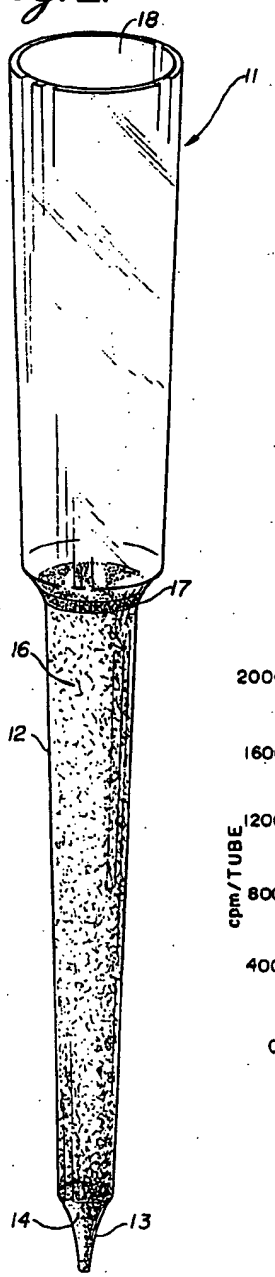
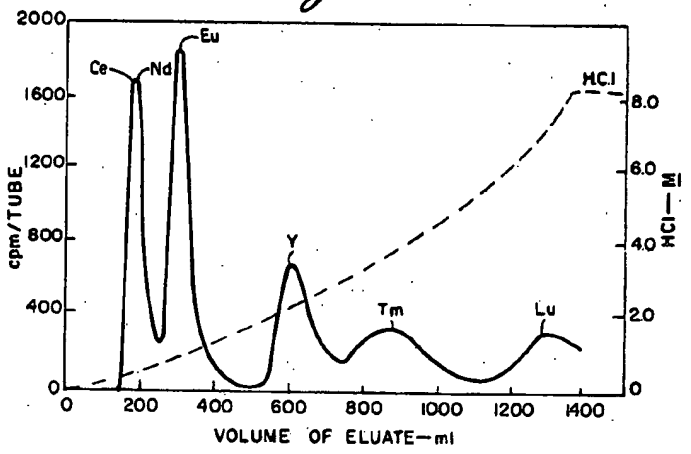


Fig. 2.



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United States Patent Office 3,478,886
Patented Nov. 18, 1969

3,478,886

GRAFT-COPOLYMER COLUMN SUPPORT MATERIAL FOR LIQUID-LIQUID PARTITION CHROMATOGRAPHY

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Filed Mar. 31, 1967, Ser. No. 628,249
Int. Cl. B01d 23/10
U.S. Cl. 210—198 9 Claims

ABSTRACT OF THE DISCLOSURE

A graft copolymer chromatography column substrate material or column packing material, comprising polytrifluorochloroethylene powder having copolymerized and crosslinked polyethylene glycol and styrene chemically bonded to the powder particle surfaces to confer upon the material hydrophilic and organophilic properties for improved retention and transmission of organic and aqueous eluant phases in chromatographic columnar and non-chromatographic liquid-liquid partitions.

Background of the invention

The present invention relates to liquid-liquid partition chromatographic column support media in the form of dense chemically inert polymer particles having a copolymerized mixture of monomers, having hydrophilic and organophilic properties, attached by valence bonds grafted to the surfaces thereof.

Liquid-liquid extraction is a technique which has been employed for decades for separating chemical species. Today, extensive compilations of solubility data and distribution coefficients are readily available in the literature. Since, for any two distinct chemical species, regardless of similarity, there exists one or more solvent pairs for which the distribution coefficients of these species are different, coupled with the ready access to such data in the literature, liquid-liquid extraction is today a most versatile tool for separating compounds in solution.

The basic approach to liquid-liquid extraction is, of course, to thoroughly mix two or more solvent phases including the chemical species to be separated in a conventional separatory funnel so that there is a differential partition of chemical species between the phases, and to physically separate the phases having different chemical species or concentrations thereof to provide a separation of such species. If the distribution coefficients of the species are sufficiently different, they can be separated in a few successive extractions which are easily carried out in a separatory funnel. However, for separating compounds which have similar characteristics and accordingly similar distribution coefficients, an undue number of successive separations is required which renders the use of separatory funnels impractical.

Description of the prior art

Various automated flow systems have been developed which are capable of satisfactorily fractionating mixtures of various compounds including those very similar solvent properties. Another approach to the problem is to utilize what has become known as reverse phase partition chomatography. In this process, one liquid phase is absorbed onto a substrate support in a chromatographic column, forming a more or less stationary liquid film, and then

another liquid, the mobile phase, is transported in progressive contact past the stationary phase. The different chemical species are continuously redistributed between the mobile and liquid phases, and become separated because the individual species have different distribution properties between the phases and therefore progress through the column at different velocities, e.g., in the order of their relative affinities for the mobile phase. The theory and detailed mechanism of reverse phase partition chromatography is described in the literature, e.g., in an article entitled "Chromatography," Encyclopedia of Chemical Technology, published by Interscience Publishing Company, page 413.

The packing material which serves as substrate for adsorption of the stationary phase is generally a substance which has a high affinity for one of the extraction liquids and not the other. One packing material commonly used is a finely divided fluorocarbon polymer. By their very nature, i.e., finely-divided particulate loading, the columns offer a high impedance to the flow of the mobile phase. To overcome this impedance, the mobile phase may be forced through the column under pressure, e.g., by the use of compressed air in the headspace in the mobile phase liquid reservoir. It is this circumstance, i.e., that the mobile phase must be pressurized, which provides a major source of trouble which limits the usefulness of columnized liquid-liquid extraction. For example, pressurization requires a host of auxiliary equipment, such as gas pressure reservoirs, pressure lines, leak tight connections, manometric equipment and the like, which is troublesome, per se, in operation. Far more serious, however, is the danger that all or part of the column will run dry, which occurs when the level of the mobile phase falls below the column entrance. Since this destroys the requisite separation conditions in the column, it is always necessary to monitor the liquid level in some manner, leading to additional complication. Moreover, if more than one mobile phase fluid or eluant are necessary in the separation, all problems are multiplied and the procedure becomes increasingly cumbersome and time-consuming. Accordingly, practical use of reverse phase partition chromatography, a potentially versatile and effective procedure, has been limited almost exclusively to separating substances having very similar distribution coefficients, and preferably with separation schemes employing a minimal number of eluant phases, since the tedious and time-consuming nature of the operation cannot otherwise be economically justified.

There are also known in the polymer art, although not in association with column substrates for liquid-liquid extractions, certain graft copolymers which consist of two or more monomer types having hydrophilic and hydrophobic or organophilic character. These substances have the rather interesting property that they interact with both organic and polar liquids. However, these materials have not in the past been particularly useful as packing materials for chromatographic columns due to their low chemical stability and high solubilities in certain solvents.

I have attempted to enhance the chemical stability of graft polymers having mixed hydrophobic and hydrophilic functional groups by crosslinking such compounds, e.g., by polymerization under the influence of ionizing radiation and/or suitable initiators. While I have found that the polymers so treated are indeed able to withstand the action of organic and inorganic fluids of wide pH ranges, their performance as column substrates has been

disappointing because the concentration of the various chemical species discharged in the effluent decreases too slowly with time from a maximum to zero. This condition is known in the art as "tailing," which is descriptive of the shape of the output concentration vs. discharge time graph. Ideally, the concentration of any species fed into the column should rise abruptly to a maximum and drop back to zero quickly at a distinct point in the liquid flowing through the column. Due to the tailing phenomenon noted above, successive output peaks overlap, which overlapping, i.e., intermixing, comprises the purity of the individual species collected. Although not completely understood, it is believed that the highly convoluted microstructure of the crosslinked materials traps some of the solute molecules and releases them only slowly to the mobile phase. In any event, such crosslinked materials have not been found especially suitable as liquid-liquid partition chromatographic column substrates.

Accordingly, a principal object of the present invention is to provide a support substrate for liquid-liquid partition chromatographic columns which is capable of absorbing the stationary liquid phase, and will offer little or no resistance to the flow of the mobile phase.

Another object of the invention is to provide a substrate material which is chemically inert, is insoluble in a wide range of organic and inorganic solvents, including strong acids and bases, and which will not give rise to the condition known as "tailing."

A still further object of the invention is to provide a method for rapidly carrying out a columnar liquid-liquid partition separation of solute species which differs markedly in their distribution coefficients.

These and other objects will become apparent upon consideration of the following specific description, in conjunction with the drawings, in which:

FIGURE 1 represents a preferred liquid-liquid extraction column for separating substances which differ substantially in their distribution coefficients; and

FIGURE 2 is a graph of various rare earth elution peaks obtained by separating the rare earth elements by means of liquid-liquid extraction through the present substrate materials.

Summary

A principal aspect of the present invention is in the provision of a unique family of graft copolymers which comprise a base of dense macro particles of a polymeric, relatively inert material, such as polytetrafluoroethylene and polytrifluorochloroethylene which is modified in its chemical and surface properties by the provision of selected polymeric functional substituent sidechains which are chemically bonded to the surface of the dense base particles to provide the requisite properties. As used in the context of the present application, with reference to the nature of the base material, the term "macro particle" is used to indicate that the base material is not present in a stoichiometric adsorptive or reactive quantity, but rather is in the form of a dense solid particle having an impermeable solid core polymeric resin material. The sidechain substituents are attached to the surface of the core of the substrate particle, rather than to any particular position on the molecules making up the core. The size of the "macro particles," while not especially critical, generally ranges between 100 and 325 mesh. The term "dense" is used to denote the property of being relatively impermeable to solvents, be it due to the crystalline structure of the material, close interatomic distances, or electrical properties. This bulk property can be empirically determined by the degree of swelling of the base material in solvents, which degree of swelling should be well below about 1% by weight.

The sidechain substituents comprise at least two principal types of molecular fragments, e.g., one type bearing functional groups which are adsorptive with respect to one of the liquid phases in the extraction system, e.g., a non-polar organic phase, and another type bearing functional groups which attract the second extraction liquid, which may be a polar liquid, such as water. Throughout the present application, the term hydrophilic is used, since, as a practical matter, in the overwhelming majority of liquid extractions, water is used as the eluant phase. It is to be understood, however, that any non-aqueous eluant could be used as well, together with the appropriate sidechain material having wetting characteristics with respect to this eluant. These molecular fragments are copolymerized with the substrate material and also among themselves, in an intimately intermixed order, both types of sidechains being bonded to the surface of any one base particle in order to offer both liquid phases a continuous maze of adjacent sites capable of attracting or having selective affinities for particular solute materials extending throughout the bulk of the packing material as assembled in a column. I have found that, by virtue of this bulk structure of a quantity of the material, the present graft copolymers are capable of firmly adsorbing several immiscible liquids. Still more important, both type liquids flow freely and with little hindrance through the interstices of the substrate material. Thus, a column packed with the present surface grafted polymeric particulate substrate, saturated with the liquids, can effectively transmit solvent fluid or fluid mixtures merely by the force of gravity alone, e.g., without pressurization of any kind, and without running dry. It is this property which makes the present graft copolymer packings ideally suited for liquid-liquid partition or extraction chromatography.

In principle, the class of substituents which may be utilized to produce the particle surface grafted copolymers outlined in the above description encompasses essentially selected polymerizable monomers having the required solvent-attracting properties and capable of undergoing a graft copolymer forming reaction, with the particle base polymer being any of those suitable for use with or compatible with the solvents to be employed in the extraction process. In general, fluorinated polymers such as polyfluoroethylene or polyhalo polymers such as polytrifluorochloroethylene are preferred, since they are insoluble in a large number of solvents. Under less demanding conditions, polyethylene, polypropylene, polyvinylchloride or the like might be employed. Specific materials useful for any one liquid-liquid extraction are thus ultimately determined by the solvent-liquids of the stationary and eluant phases used in the extraction procedure. Given the solvents, one can thus choose an appropriate base material and sidechain constituents from the comprehensive tabulations of requisite properties avilaable in the literature. Some common solvents and appropriate materials for synthesizing a packing material useful for each are given in the table below.

TABLE I

Solvent	Polymer type (for sidechains)
Water and water miscible alcohols.	Poly(ethylene glycol). Poly(vinyl alcohol). Poly(acrylic acid). Polyacrylamide. Starches, dextrins and other polysaccharides. Cellulose.
Dimethylformamide	Polyacrylonitrile.
Dioxane	Poly(methyl methacrylate).
Carbon disulfide	Poly(methyl acrylate).
Acetonitrile	Poly(ethyl acrylate).
Nitromethane	Poly(vinyl acetate).
Tetrahydrofuran	Polystyrene.
Ethyl acetate	Poly(n-butyl methacrylate).
Methyl ethyl ketone	Poly(isobutyl methacrylate). Poly(n-butyl acrylate).
Diisobutyl ketone	Poly(n-butyl methacrylate).
Diethyl ether	Poly(isobutyl methacrylate). Poly(n-butyl acrylate).
Cyclohexane	Polystyrene.
Benzene	Polydimethyl siloxane.
Toluene	Poly(methyl methacrylate).
Carbon tetrachloride	Poly(methyl acrylate).
Chloroform	Poly(ethyl acrylate).
Dichloroethane	Poly(vinyl acetate). Poly(n-butyl methacrylate). Poly(isobutyl methacrylate). Poly(n-butyl acrylate).

The weight relationships of the sidechain and base particle constituents are determined by the weight increase of the base polymer after grafting and thorough separation of the grafted product from the unreacted residue. The total percentage of both grafted sidechain constituents should be between about 2% by weight to about 18% by weight of the total. These weight ratios are not too well defined. In the lower percentage region, e.g., a sidechain content of up to about 5% by weight, the bulk behavior of the material improves progressively, both with respect to the quantity of stationary phase retained and the flow rate of eluant passing through the graft. The optimum behavior of the column packing material is between about 10 and 12% by weight of sidechain constituents. At around 18 to 20% by weight, the "tailing" problem referred to above sets in, which is thought to be due to the increased material thickness and volume occupied by the sidechain constituents, which appears to become large enough to entrain and delay some of the eluate.

Since the determination of the weight ratio of hydrophilic and organophilic sidechains is very difficult to determine by analysis of the graft copolymer, it is preferable to state this weight relationship in terms of their concentration in the reaction mixture prior to copolymerization, although the amounts ultimately grafted to the substrate depends on a number of variables, as further discussed below. On the basis of the starting concentrations the ratio between the hydrophilic and organophilic material is between about 1:3.5 and about 1:1. As determined by material balance methods, a very satisfactory column material has a ratio of about 1:1.5 of grafted hydrophilic and organophilic sidechains.

From the materials given in the table and on the basis of state of the art knowledge regarding compatibilities and behavior of chemical compounds, a large class of packing materials can be synthesized, each one of which being adequate for use with some specific solvent pair. For example, for the common extraction system, dimethylformamide (DMF)-water, where DMF is the usually stationary phase and water the eluant phase, a medium having a mixture of polyacrylonitrile and polyacrylamide copolymerized and grafted as a surface layer upon polytetrafluoroethylene particles could be employed. The polyacrylonitrile sidechains serve to adsorb the stationary DMF phase, whereas the polyacrylamide portion of the graft copolymer attract and guide the aqueous phase. The method of synthesizing these graft copolymers is discussed in detail below. While this graft copolymer is an excellent support for the DMF-water system, it should not be used at extreme pH values, due to the fact that the sidechain materials tend to hydrolize.

Many of the combinations of sidechain materials and base materials are highly specific, in the sense that they may work well with a few solvent systems, but not with others.

However, I have also developed a preferred substrate material which can be used with a wide variety of solvent systems. This preferred substrate material comprises a base of polytrifluorochloroethylene in the form of a powder of a size between 100 and 325 mesh. In the preferred substrate, the sidechains which are grafted to the base particle surfaces are comprised of polyethylene glycol and polystyrene and copolymers thereof, hereinafter referred to as poly(ethylene glycol-g-styrene). The polymeric composite particle substrate obtained by the foregoing procedure is attacked only by very caustic chemical agents, such as powerful oxidizing agents in strong acid solution by virtue of the complete absence of reactive functional groups or other reactive sites therein. Thus the substrate material can be used universally in extraction chromatography without degradation, i.e., even with highly acidic and basic media.

The polyethylene glycol constituents are hydrophilic, and have an affinity for polar liquids and solvents generally, whereas the polystyrene chain portions have an affinity for and attract organic and non-polar solvents and liquids. By virtue of the impermeability of the polytrifluorochloroethylene base particles to the solvent or liquids in contact therewith, the effective interstitial space accessible to solvent interaction in the bulk of the material is limited to sites situated between the grafted adjacent sidechain layer on the surface of the particles. The eluant phase is held in these interstitial spaces by the adsorptive properties of the sidechains and by capillary action, providing a persistent superficial layer of fluid therein. Accordingly, while excess liquid of either type will freely migrate through the bulk, displacing the adsorbed fluid along the sequence of sites which attract it in accord with usual chromatographic column operation, the substrate does not run dry since at least a persistent surface layer of fluid remains imbibed in the grafted copolymer layer. On the other hand, this layer of grafted copolymer which is provided on the surface of the substrate particle is of a thickness and of a structure such that rapid interchange with little delayed holdup occurs on contact with the partition solvent system, so that rapid and efficient separation or adsorption of solutes occurs without undue "tailing."

Synthesis of column support materials

In general, the crucial step in the synthesis of the present column packing materials is the copolymerization and grafting of the ingredients of the surface layer of the packing material, since it ultimately determines the nature in which the material is linked. For example, if it is desired to provide a bulk material having the majority of hydrophobic and hydrophilic sidechains attached to the base particles individually, these polymers are produced separately and a mixture of both polymer species is then grafted to the base particles by irradiating a mixture thereof, including some vinylic monomers, for example. The polymerized product is then washed to remove ungrafted particles to produce a product with a substantial fraction of individual sidechains of either the hydrophobic or hydrophilic types and, of course, some copolymerized sidechains.

As indicated above, the preferred packing material is comprised of an inert polymer base particle bearing a surface layer of copolymerized hydrophilic and hydrophobic sidechain constituents, since in this structure, hydrophilic and organophilic sites are more intimately dispersed which enhances the ability of the resin bulk to hold and transmit the liquid phases. To synthesize such a material, monomeric or prepolymerized precursor sidechain material of selected types is copolymerized to adhere to the surface of the base powder particles by exposure to ionizing radiation.

Since the preferred base material is generally chemically unreactive, i.e., not subject to catalytically induced polymerization, the ultimate graft of the sidechain substituents of the invention is preferably accomplished by irradiation of a mixture of the base material particles in contact with the sidechain polymer. General requirements of radiation polymerization processes, per se, are well known and are discussed in detail in the literature. More particularly, an intimate mixture of selected prepolymerized materials, partially polymerized materials, monomers, and mixtures thereof, together with crosslinking agents and suitable solvents, if appropriate, is first prepared.

The reaction mixture is then irradiated with a dose of about 10,000–10^6 rads. Subsequently, the polymerized reaction product is separated from the mixture by filtering and washing the precipitate with suitable solvents for removing solvent, low molecular weight polymer, unpolymerized monomers, etc., and drying.

The values of two typical polymerization runs are given below in Table II.

TABLE II

Irradiation No.	808-8	808-9
Mesh size of Kel-F Powder	−325	250-325
Wt. of Kel-F Powder (grams)	100.0	70.0
Wt. of Carbowax 20M	10.5	8.4
Wt. of styrene monomer	21.0	16.8
Dose (rads)	400,000	400,000
Irradiation time (hours)	48	48
Wt. of modified Kel-F product [1]	112.5	78.8
Wt. of unreacted Carbowax	6.4	5.1
Wt. of unreacted styrene	0.1	4.0
Wt. of soluble graft copolymer [2]	6.5	7.3
Wt. of Carbowax on Kel-F Powder [3]	3.8	3.0
Wt. of polystyrene on Kel-F Powder [3]	8.7	5.8
Polystyrene/Carbowax weight ratio	2.3	1.9

[1] After thorough washing and drying.
[2] Chemical analysis: 4.6 Carbowax, 95.4% polystyrene. Includes polystyrene homopolymer produced in reaction.
[3] By difference.

The irradiation time and dose rate have, of course, some influence upon the chainlength of the grafted materials, the degree of copolymerization among the sidechains themselves, as well as the total amount of sidechain material grafted to the base powder. Accordingly, an increase in the irradiation parameters will result in a corresponding change in each of these properties, i.e., chainlength, degree of copolymerization, and base particle weight increase.

EXAMPLE I

A batch of a preferred packing material of poly (ethylene glycol-g-styrene) modified polytrifluorochloroethylene was prepared by dissolving 20 g. of polyethylene glycol (Carbowax 20M, Union Carbide Company), and 40 ml. of styrene in 40 ml. of methanol. The resulting solution was then mixed with about 200 g. of polytrifluorochloroethylene powder, irradiated with fission product gamma rays (from a spent reactor fuel element) at a rate of about 30,000 rads/hr. for about 20 hours. After irradiation, the solution was filtered and ungrafted styrene and Carbowax polymers removed from the filtrate by washing the insoluble polymer with toluene. The residue, i.e., the substrate particle bearing a surface layer of grafted copolymer, was then washed in methanol to remove toluene and dried.

Other exemplary graft copolymer substrate formulations especially suited for the indicated extraction solvent system are the following:

EXAMPLE II

For water-dioxane extractions:

	Percent by weight
Polyvinyl alcohol	25
Vinyl acetate monomer	25
Polytrifluorochloroethylene powder	50

EXAMPLE III

For alcohol or water-methyl ethyl ketone extractions:

	Percent by weight
Polyacrylamide	15
n-Butyl alcohol monomer	35
Polytetrafluoroethylene powder	50

EXAMPLE IV

For water-cyclohexane extraction:

	Percent by weight
Polyacrylic acid	20
Dimethyl siloxane monomer	30
Polymeric amide (nylon) powder	50

EXAMPLE V

For water-chloroform extractions:

	Percent by weight
A water soluble starch	25
Ethylacrylate monomer	25
Polypropylene powder	50

Each of the components given in Examples II–V are mixed, copolymerized by radiation, washed and dried, as indicated in Example I.

EXAMPLE VI

A group of rare earths was separated by passing a dilute H-Cl solution of these rare earths through a column packed with the substrate material of Example I. The active portion of stationary phase was the organic complexing agent di-(2-ethylhexyl)orthophosphoric acid. The output of the column is shown in FIGURE 2. The eluant was passed through the column without the application of pressure or force of any kind. The absence of "tailing" is evidenced by the sharp drop-off of the neodynium and europium peaks.

Preparation of columns

In general, chromatography columns are packed with the present substrate in a conventional manner, i.e., the graft copolymer particulate packing medium is stirred with the liquid which is to be used as the stationary phase, e.g., generally an organic complexing agent or a solution thereof. After thoroughly soaking the packing medium in the stationary phase liquid, it is stirred into a bath of the material used for the mobile phase, which is usually water or an aqueous phase. The thoroughly wetted graft copolymer is then carefully packed into an elongated tubular column, preferably between two thin layers or plugs of a porous material such as glass powder or glass wool at the top and bottom ends of the column. In accord with usual practice, the packing material should be emplaced carefully in small quantities at a time in the column, making sure that the column is packed uniformly without void spaces by gently pressing the medium together.

In addition to providing a packing material for conventional columns for liquid-liquid partition of the type used in the separation of species having similar distribution coefficients, another important feature of the present invention is a column of the type depicted in FIGURE 1. This column is primarily intended for rapidly separating chemical species which differ appreciably in the magnitude of their distribution coefficients, i.e., by at least 20%, and comprises a generally funnel shaped glass envelope **11**, the stem portion of which defines a relatively short tubular column **12** which terminates in a tapered tip **13**. The tip retains a conical plug **14** of fritted glass or a similar porous inert material. The remainder of the column is packed with a column support material **16** comprising one of the graft copolymers disclosed hereinabove, as for example polyethylene glycol-g-styrene modified polytrifluorochloroethylene. A thin layer of glass powder **17** or the like is placed on top of the column support material for protection. The top end **18** of the glass column is flared to provide a bowl-shaped receptacle for solution to be separated.

The extraction column shown in FIGURE 1 is used in place of a separatory funnel for separating species which have markedly different mobilities through the column. The column packing medium is prepared as previously, and the solution of the chemical species transferred into the top end **18**. The high mobility chemical species is eluted rapidly and is collected with the eluant in a suitable receptacle placed beneath the funnel. The slow moving species are retained in the column in the superficial interstitial fluid volume.

The principal advantages of this separator are its low cost, allowing it to be made as a disposable item, and also its small size and low space requirement, and that it does not have to be shaken or agitated. Thus, for use as in a conventional sealed glove box, the storage and use of as few as 12 separating funnels is exceedingly problematical, as many as 100 separators of the type described can easily be accommodated and effectively used.

It will be readily realized that a number of the present separators may be prepared with different absorbed or stationary phases and used successively to eliminate different species as necessary for the isolation of a desired fraction or further resolution of eluates obtained in previous separations. It is also possible to provide a

suitable flow control device at the end of the column, for example, to adjust the flow velocity of the mobile phase.

I claim:

1. A packing medium for use in liquid-liquid partition chromatography and providing a minimized tailing characteristic comprising:

(a) dense impermeable inert substrate base particles of a fluorocarbon polymer selected from the group consisting of polytetrafluoroethylene and polytrifluorochloroethylene, said particles having a particle size in the range of about 100 to 325 mesh;

(b) a mixture of hydrophilic and organophilic polymeric constituents in an amount in the range of about 2 to 18% by weight copolymerized and grafted so as to be attached by valence bonds as a layer disposed upon the surface of said particles,

said hydrophilic constitutent being a material selected from the group consisting of polyacrylamide, starch, polyacrylic acid, polyethylene glycol, polyvinyl alcohol and celluose,

said organophilic constituent being a material selected from the group consisting of polyacrylonitrile, polystyrene, polydivinylbenzene, polymethylmethacrylate, polydimethylsiloxane, polymethyl acrylate, polyethyl acrylate, polyvinyl acetate, poly(n-butyl methacrylate), poly(isobutyl methacrylate), and poly(n-butyl acrylate),

said constitutents being derived of precursor material having a weight ratio in the range of about 1:1 to 1:3.5, hydrophilic to organophilic, respectively.

2. A packing medium as defined in claim 1 wherein said substrate base particles have a degree of swelling in said liquids below about 1% by weight.

3. A packing medium as defined in claim 1 wherein said hydrophilic and organophilic are copolymerized and grafted on the surface of said particles by exposure to an irradiation dose in the range of about 10^4 to 10^6 rads of gamma radiation.

4. The packing medium of claim 3, further defined in that the combined weight of said hydrophilic and organophilic constituents is between 10 and 12% by weight of said substrate base particles.

5. The packing material of claim 3, further defined in that the weight ratio of said hydrophilic to organophilic constituents is about 1:1.5, respectively.

6. A packing medium as defined in claim 1 wherein said hydrophilic constituent comprises polyethylene glycol and said organophilic constituent comprises polystyrene wherefor the surface layer comprises ethylene glycol-g-styrene copolymerized and grafted to the surface of said particles.

7. A packing medium as defined in claim 6 wherein the combined weight of said polyethyleneglycol and polystyrene constituents is in the range of about 5% to about 12% of the weight of the substrate particles.

8. A gravity flow liquid-liquid partition chromatography apparatus for separating chemical species having distribution coefficients differing by at least 20% comprising:

(a) an elongated generally cylindrical tubular envelope of an insert material, said envelope defining an elongated tubular lower stem portion and an upper flared receptacle bowl portion communicating with said stem portion,

(b) a packing medium disposed in the stem portion of said envelope, said medium comprising substrate particles having a size in the range of about 100 to 325 mesh of a fluorocarbon polymer selected from the group consisting of polytetrafluoroethylene and polytrifluorotrichloroethylene having polyethylene glycol and polystyrene in a weight ratio in the range of about 1:1 to about 1:3.5, respectively, copolymerized and grafted on the surface of said particles in an amount in the range of about 5 to 13% of the weight of said particles; and

(c) porous plug means disposed within the lower end of said stem to retain said medium therein.

9. The apparatus of claim 8, further defined

in that said stem portion terminates in a tapered annular outlet tip, and

in that a layer of inert powdered material is disposed to cover the upper surface of the medium in said stem portion.

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JAMES L. DECESARE, Primary Examiner

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204—159.17

United States Patent [19]

Marteau D'Autry

[11] **Patent Number: 4,766,082**

[45] **Date of Patent: Aug. 23, 1988**

[54] **METHOD AND APPARATUS FOR PREPARING SAMPLES FOR ANALYSIS**

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[22] Filed: **Aug. 26, 1987**

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[30] **Foreign Application Priority Data**

Oct. 24, 1984 [FR] France 84 16283

[51] **Int. Cl.**[4] **G01N 1/18**; G01N 35/02

[52] **U.S. Cl.** **436/178**; 436/47; 436/161; 422/59; 422/65; 422/70; 422/101; 210/198.2; 210/287; 73/863.32

[58] **Field of Search** 436/47, 161, 178; 422/59, 65, 70, 101; 210/198.2, 287; 73/863.32

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[57] **ABSTRACT**

The present invention relates to a method of preparing samples for the purpose of analysis, in which at least one reaction agent and an initial sample are successively injected into a cartridge (90) provided with an orifice (92) in its lower portion, in such a manner as to cause the injected matter to pass through a column (91) of powdery agent contained within the cartridge, and in which the product of the last passage of injected matter through the column is retained for analysis. According to the invention, the reaction agents and the initial sample are successively injected into each cartridge after the top of the cartridge has been sealed in such a manner that the injection of the reaction agent or the sample causes the pressure at the top of the cartridge to rise, thereby accelerating the passage of the reaction agent or the sample through the column. The invention also provides apparatus for performing the method, which apparatus includes a moving carriage (120) enabling a waste receptacle (130) or individual tubes (150) to be placed beneath the lower orifices of the cartridges.

6 Claims, 4 Drawing Sheets

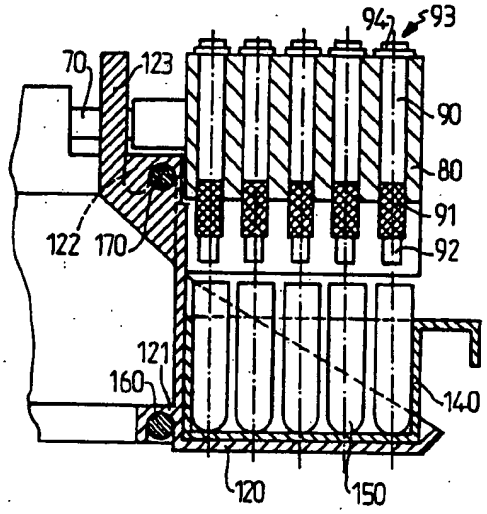
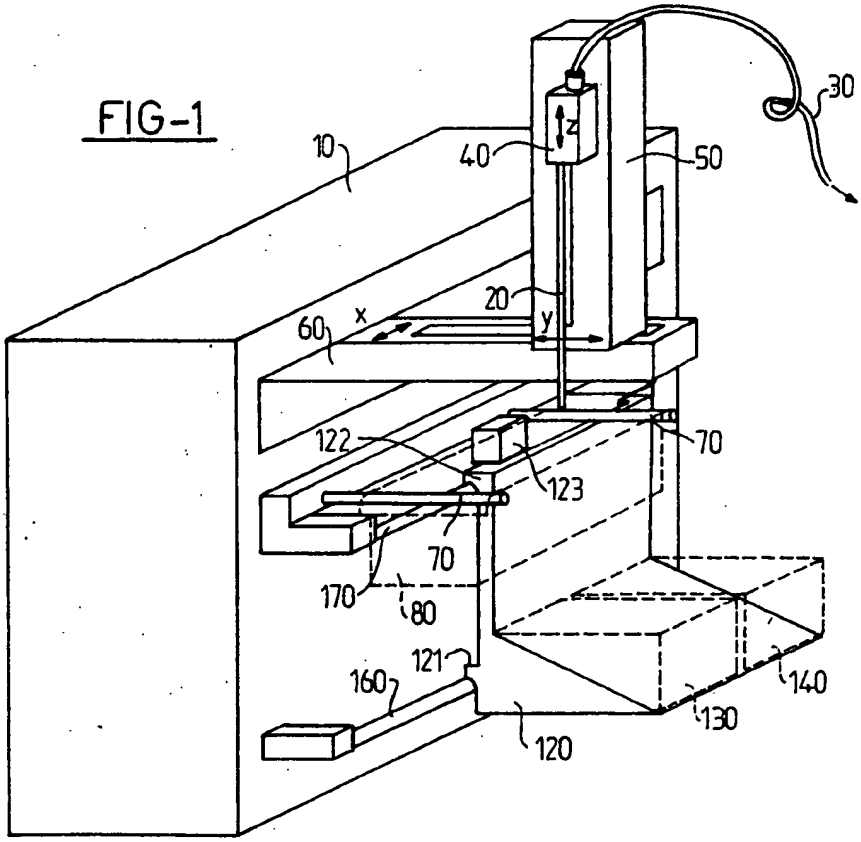


FIG-1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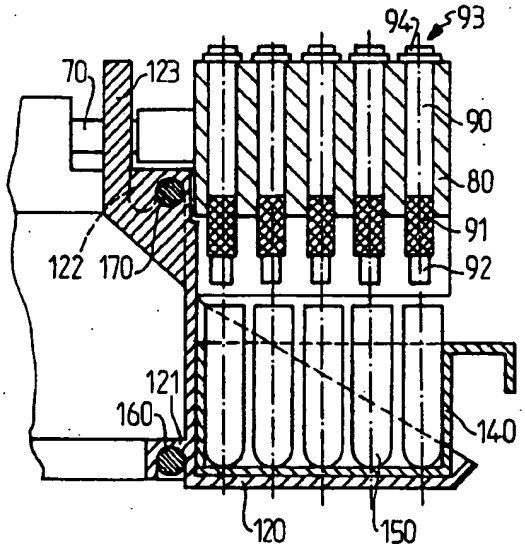
120

FIG-2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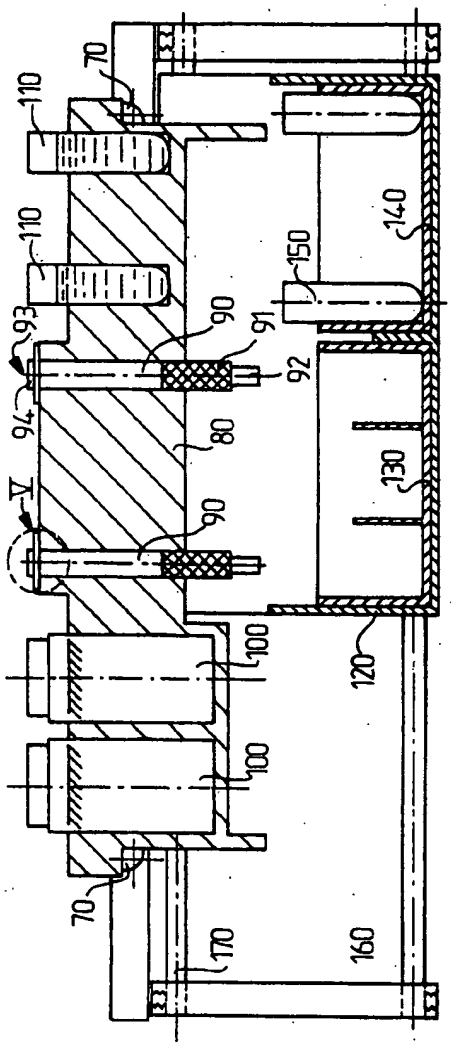

FIG-3

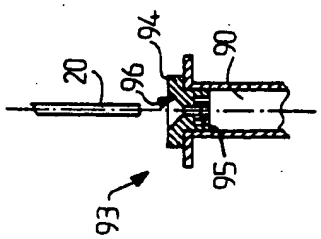

FIG-5

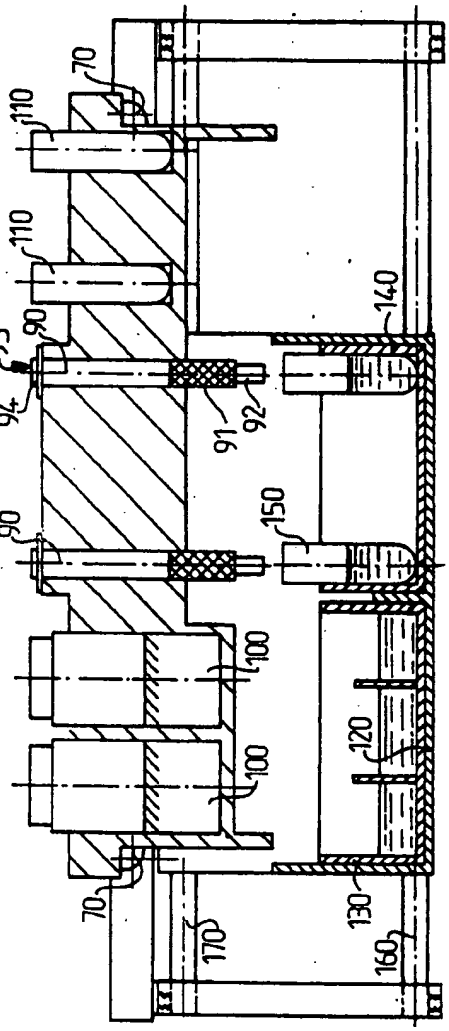

FIG-4

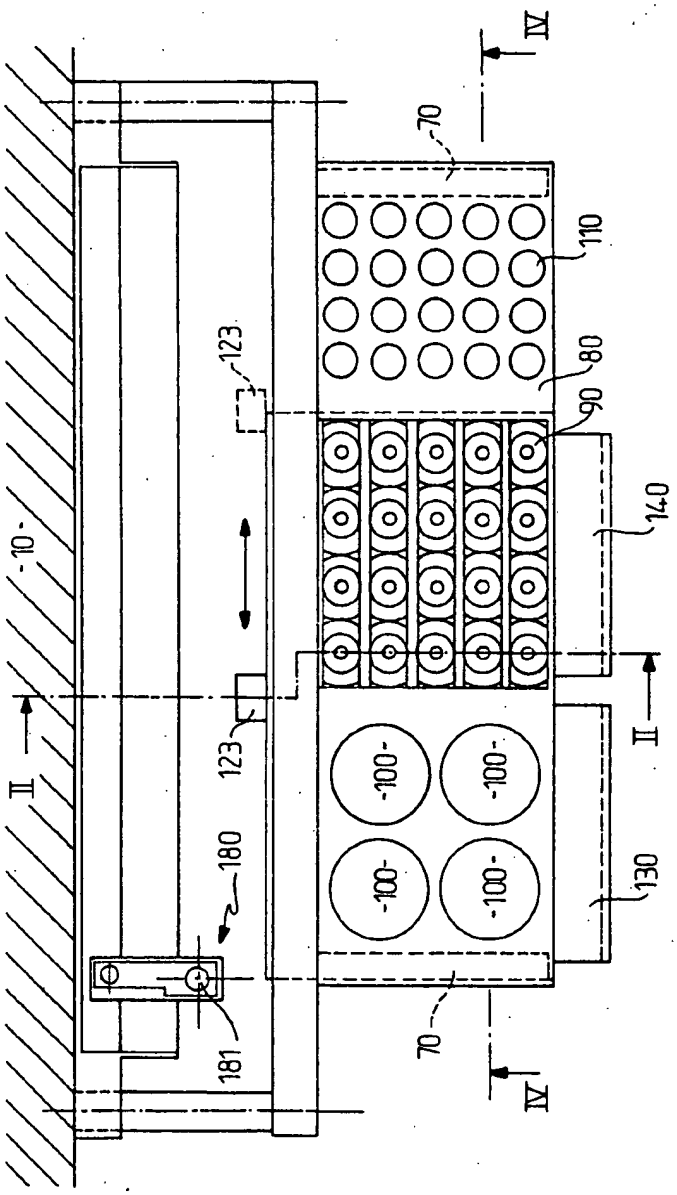
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FIG-6

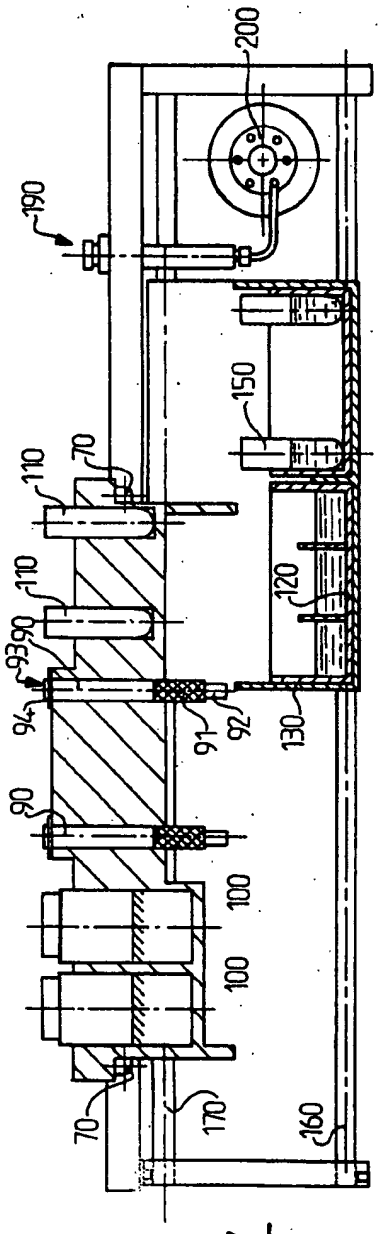
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FIG-7

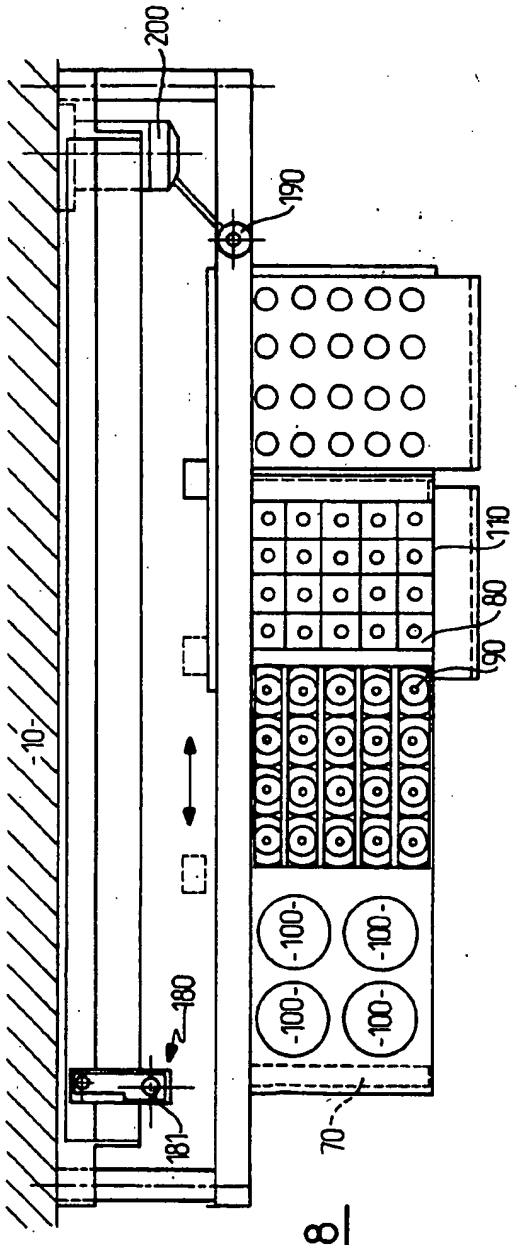
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FIG-8

METHOD AND APPARATUS FOR PREPARING SAMPLES FOR ANALYSIS

This application is a continuation of Ser. No. 790,442, filed Oct. 23, 1985, now abandoned.

The present invention relates to a method and to apparatus for preparing samples to be analyzed.

BACKGROUND OF THE INVENTION

The invention relates to a method of preparing samples in which at least one reaction agent and an initial sample are successively injected into a cartridge containing a column of powdery agent in such a manner as to cause the injected matter to pass through the column. The cartridge also has a bottom outlet orifice, and the product of the last passage through the column is collected separately and constitutes the final sample for analysis.

The reaction agents may be injected before or after the initial sample is injected. When injected before, the reaction agents serve to impregnate the column prior to the sample being injected; when injected after, they react with a column which has already been impregnated with the sample (and optionally, with other reaction agents).

The products of most of the passages through the cartridge are rejected: only the product of one specific passages is collected for analysis purposes. If the cartridges are reusable, the last material injected may be a cleaning or reconditioning agent.

One of the drawbacks of such a method lies in the time required for the sample and the reaction agents to pass through the column which they are to impregnate.

In order to accelerate such passages, suction means are generally connected in sealed manner to the bottom outlet orifices of the cartridges. The reduced pressure set up in this manner increases the speed at which injected material passes through the column and thus reduces the total time required for preparing a sample to be analyzed.

However, such suction means are bulky devices which need to match the size of the cartridges used and which need to be used in conjunction with a vacuum pump.

Preferred implementations of the present invention provide a method which reduces the total time required for preparing samples for analysis, and which avoids the need for suction means; the apparatus is thus simplified and does not require an additional source of energy such as required by a vacuum pump, and it is easily adapted to sample-preparing apparatus already in existence.

SUMMARY OF THE INVENTION

To this end, in accordance with the invention, the reaction agents and the initial sample are successively injected into a cartridge whose top has previously been closed in sealed manner so that injecting the reaction agent or the sample causes a pressure increase at the top of the cartridge suitable for accelerating the passage of the reaction agent or the sample through the column.

Preferably, immediately after injecting the reaction agent or the sample, additional air is also injected so as to increase the previously established excess pressure.

The invention also provides apparatus for performing the method.

This apparatus is of conventional type, sometimes known as an "automatic sample preparer", i.e. it is an apparatus of the type comprising:

first support means for at least one reaction agent receptacle, for a plurality of initial samples, and for a plurality of cartridges; and

a sampling and injection needle movable over the first support means and suitable for sucking predetermined quantities of a given reaction agent or of a given initial sample, and for ejecting the sucked quantity into a predetermined cartridge.

According to the invention, the apparatus further comprises:

means providing sealed co-operation between each cartridge and the needle, for closing the top of each cartridge in sealed manner after the needle has penetrated therein; and

second support means located beneath the first support means, and suitable for supporting tubes or recesses disposed beneath the bottom outlet orifices the cartridges, said tubes or recesses being suitable for individually collecting for subsequent analysis the products of the final passes of injected material through respective columns.

Advantageously, the second support means are selectively movable as a function of the steps of the method between a first position and a second position in such a manner that:

in the first position a waste collector receptacle carried by the second support means is placed beneath the cartridges to recover the products of passage through the cartridges during at least one early step of the method; and

in the second position individual tubes carried by the second support means are placed beneath the respective cartridges to recover the products of the final step passing through the cartridges.

Advantageously, the second support means are provided with a control lug, and the moving needle engages said control lug in such a manner as to drive said second support means and to cause them to pass from one of said positions to the other position.

Changing position is thus easily performed by an additional movement of the needle without requiring special drive means to be provided for the second support means. However, other drive means for the second support means could be provided (e.g. an independent motor of any suitable type).

BRIEF DESCRIPTION OF THE DRAWINGS

An embodiment of the invention is described by way of example with reference to the accompanying drawings, in which:

FIG. 1 is a perspective view of an apparatus in accordance with the invention;

FIG. 2 is a cross-section through said apparatus on a line II—II of FIG. 6;

FIGS. 3 and 4 are front views of the apparatus on a section line IV—IV of FIG. 6, showing the apparatus respectively during the preliminary steps and during the final step of the method;

FIG. 5 shows a detail marked B of FIG. 3 and illustrates the structure of the top of a cartridge; and

FIG. 6 is a plan view of the apparatus.

FIGS. 7 and 8 are views similar to the views of FIGS. 3 and 6, corresponding to an embodiment of the apparatus, in which the second support means may be set to a

third position where the samples in the tubes can be drawn for analysis.

MORE DETAILED DESCRIPTION

In the figures, reference 10 designates an automatic sample preparing apparatus of known type, for example a GILSON model "221" or "222" machine. This apparatus includes an injector needle 20 connected by a duct 30 to a suction device such as a GILSON "401" type diluter.

The needle 20 is movable along three directions X, Y, and Z under the control of moving arms 40, 50, and 60. X-Y displacement serves to place the needle over a series of tubes or receptacles capable of being selected automatically. Z displacement serves to insert the needle into the selected tube or receptacle in order to suck liquid therefrom or to inject liquid thereto, with the needle then being raised prior to displacement to some other position.

The various tubes or reaction agents (visible in the plan of FIG. 6) are supported on horizontal bars 70 which receive a support 80 which is fitted with housings for the various tubes or reaction agents.

When performing the method of the invention, the support 80 houses cartridges 90, receptacles for reaction agents 100, and as many tubes containing initial samples 100 as there are cartridges.

More precisely, the cartridges 90 (which are of a type known per se) contain a column 91 of powdery agent (a silicate for example) which is impregnated by the reaction agents and the sample as successively injected during the various steps of the method. Excess injected material (i.e. that portion of the injected material which does not react with the powdery agent) flows away via an orifice 92 at the bottom of the column.

In accordance with the invention, a closing stopper 94 (see FIG. 5) is provided at the top 93 of each tube and includes an axial orifice 95 for receiving the injection needle 90 when the needle is lowered over the center of the column for injecting a reaction agent or a sample (the funnel shape 96 serves to guide the needle as it moves downwardly).

The size of the axial orifice 95 and the resilience of the stopper 94 are chosen in such a manner that when the needle has moved fully through the stopper, the top of the cartridge is hermetically sealed.

In a variant, instead of providing each cartridge with an individual stopper, the bottom of the needle could be provided with a single common stopper which would remain fixed thereto. Sealing would then be provided when the needle is lowered over a cartridge by the stopper being pressed against the top face of the cartridge.

Also in accordance with the invention, the automatic sample preparer is provided with a carriage 120 placed beneath the support 80.

The carriage has two compartments 130 and 140, the compartment 130 is used to collect the products which have passed through the columns and which are not retained; the other compartment 140 serves to support as many individual tubes 150 as there are cartridges 90 and initial samples 110, and these tubes are used to collect the products which pass through each of the cartridges during the last step of the method.

The carriage is movable in order to allow either of the two compartments 130 and 140 to be placed beneath the cartridges 90. To this end, the automatic preparer 10 further includes two horizontal and longitudinally extending slides 160 and 170 which receive grooves 121 and 122 of complementary shape in the carriage 120. The carriage is thus free to slide on the slides 160 and 170 with one degree of freedom in the longitudinal direction.

In order to slide the carriage along the slides, an operating lug 123 projects upwardly from the carriage 120 and is offset towards the housing of the sample preparer 10.

This operating lug is situated inside the zone of possible X-Y displacement of the needle 20. Thus, in order to move the carriage, the needle is moved (automatically under the control of the sample preparer) until it comes into contact with one of the side faces of the operating lug, e.g. with the left face to move the carriage to the right or vice versa. The needle is always moved longitudinally (and automatically) in the X direction when being used to drive the moving carriage.

The operation of the apparatus is now described, as are the various steps in performing the method of the invention.

By way of example, consider a method comprising the following steps:

impregnate the column with a reaction agent A;
add a reaction agent B;
add an initial sample;
add a reaction agent C;
add a reaction agent D.

During the first four steps the products which have passed through the cartridge are not conserved, they are simply collected prior to being discarded; however, during the last step the product of the passage through the cartridge is collected to constitute a final sample ready for analysis.

It should be assumed that the needle is washed between the various steps in order to avoid contamination. This operation may take place in conventional manner at a washing position 180 (FIG. 6) provided with an orifice 181 where the needle is washed.

The initial position of the moving carriage is the position shown in FIG. 3, i.e. the waste container 130 is placed beneath the cartridges 90.

The needle is then displaced over the receptacle 100 containing the reaction agent A, and a predetermined quantity thereof is sucked up. The needle is then placed over the first cartridge, is lowered, and then the reaction agent A is injected into the cartridge.

Because of the sealed closure of the top of the cartridge, the pressure therein increases during injection due to the combined volume of residual air and injected liquid.

This increase in pressure serves to blow the liquid through the column 91 towards the outlet orifice 92.

Advantageously, after injecting the liquid and without removing the needle, additional air is injected into the cartridge to further increase the pressure of the residual volume in the top of the cartridge.

Excess reaction agent flows out through the orifice 92 and is collected in the compartment 130.

After the needle has been washed, the same procedure is repeated to inject reaction agent B, then the individual sample taken from one of the tubes 110, and then reaction agent C.

Once the initial steps of preparing the cartridge have been completed, the moving carriage 120 is moved to the left (as shown in FIGS. 3 and 4) so that it occupies the position shown in FIG. 4, in which there is an indi-

vidual tube 150 located beneath each of the columns 90 in order to collect individual final samples for analysis.

The carriage is displaced, as mentioned above, by an additional movement of the needle so as to bring it into contact with the drive lug 123 and thus drive the carriage.

Reaction agent D can then be sucked up and injected into a cartridge; it is then possible, in the same manner as before, to accelerate the passage of the liquid through the column by injecting an additional quantity of air into the top volume of the cartridge.

The final sample is then obtained in the tube 150. It is then possible to restart the cycle and to prepare the next sample, after replacing the moving carriage in its initial position (FIG. 3).

In order to reduce the total time required for preparing a series of sample, the same reaction agent may be injected into a plurality of columns during any one of the steps. Such multiple injection (into five or ten cartridges) is then followed by multiple injection of air for flushing the liquid through the respective columns.

In the embodiment shown on FIGS. 7 and 8, the movable carriage 130 is allowed to be set to a third, extreme position, where the final samples collected in tubes 150 may be drawn by the needle, then injected into an injection port 190 and directed to an injection valve 200 of a chromatograph (e.g. high pressure liquid chromatography), or any other analyser.

I claim:

1. A method of preparing samples for analysis, in which at least one reaction agent and an initial sample are successively injected into a cartridge containing a column of powdery agent and provided with a bottom outlet orifice, with said reaction agent and said sample being injected in such a manner as to pass through the column of powdery agent, and in which the product of a last passage through the column is collected for analysis, the method wherein the improvement comprises said at least one reaction agent and the initial sample are successively injected into a top of a cartridge after the top of the cartridge has been sealed in such a manner substantially effective to cause an increase in pressure at the top of the cartridge by injection of said at least one reaction agent or of the sample resulting in the acceleration of the passage of said at least one reaction agent or the sample through the column substantially as a result of said increased pressure, whereby a requirement for either a vacuum pump or pressurized gas supply is eliminated.

2. A method according to claim 1, wherein immediately after injecting said at least one reaction agent or the sample, an additional quantity of air is injected into the cartridge in order to further increase said pressure at the top of the cartride.

3. Apparatus for preparing samples for analysis including:

first support means for supporting at least one reaction agent receptacle, a plurality of initial samples, and a plurality of cartridges, each cartridge containing a column of powdery agent and having a bottom outlet orifice; and

a sampling and injection needle which is movable over the first support means effective to inject predetermined quantities of given reaction agent or of given initial sample into a predetermined cartridge;

the apparatus wherein the improvement comprises:

means providing sealed co-operation between the cartridge and the needle, to provide sealed closure of the top of the cartridge after the needle has penetrated therein substantially effective to cause an increase in pressure at said sealed closure resulting from said injection, whereby a requirement for either a vacuum pump or pressurized gas supply is eliminated; and

second support means located beneath the first support means for supporting tubes or recesses disposed beneath the bottom outlet orifices of the cartridges, said tubes or recesses being suitable for individually collecting for subsequent analysis the products of the final passages through the respective cartridges.

4. Apparatus according to claim 3, wherein the second support means are movable means and are selectively movable between a first position and a second position,

in the first position the tubes or recesses carried by the second support means are placed beneath the cartridges to receive the products of passages through the cartridges during at least one early step of the method; and

in the second position, the tubes or recesses are placed beneath respective cartridges to receive the products of passages through the cartridges during a final step of the method.

5. Apparatus according to claim 4, wherein the second support means is provided with an operating lug suitable for engaging the moving needle in abutment in such a manner as to drive and move the second support means between said first and second positions.

6. Apparatus according to claim 4, wherein the second support means are further movable to a third position where the products of passages, contained in the tubes or recesses, will be drawn by the needle for injection into an analyser.

* * * * *

United States Patent [19]

Campbell

[11] **Patent Number: 4,863,610**

[45] **Date of Patent: Sep. 5, 1989**

[54] **SCRAPING, COLLECTING & ELUTING APPARATUS FOR THIN LAYER CHROMATOGRAPHY AND METHOD FOR ITS USE**

[76] Inventor: **Brian D. Campbell**, P.O. Box 2926, Kensington, Md. 20895

[21] Appl. No.: **60,402**

[22] Filed: **Jun. 11, 1987**

[51] **Int. Cl.**[4] **B01D 11/02**; B01D 15/08

[52] **U.S. Cl.** ..... **210/658**; 73/61.1 C; 210/772; 210/198.3; 422/61; 422/70; 422/101; 436/178

[58] **Field of Search** ..... 73/61.1 C, 863.23, 863.24, 73/863.25; 210/198.2, 198.3, 658, 772; 422/61, 70, 101; 436/178

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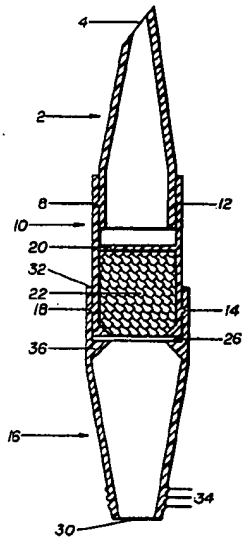
Primary Examiner—Robert Spitzer

[57] **ABSTRACT**

A device and method for scraping and collecting adsorbent from located areas on developed thin layer chromatography plates and extracting substances from collected adsorbent. The above functions can be performed without additional apparatus. The device is constructed of polypropylene to allow for easy use, manufacture and low cost. The shell surrounds a primary filtering and retaining material and a secondary filter. The adsorbent is removed from the thin layer chromatography plate via a scraping edge and collected by aspirating the adsorbent into the device where it becomes embedded in the primary filtering and retaining material. The device can then be separated to allow facile addition of a solvent to elute the sample out of the device free of adsorbent. The scraping process is improved as a result of the polypropylene contact with the plate, the collection process minimizes loss of removed adsorbent, and the elution/filtration allows easy recovery of developed samples. These functions are accomplished within this device and therefore time spent in preparation and cleanup is kept to a minimum. This device is potentially disposable.

20 Claims, 4 Drawing Sheets



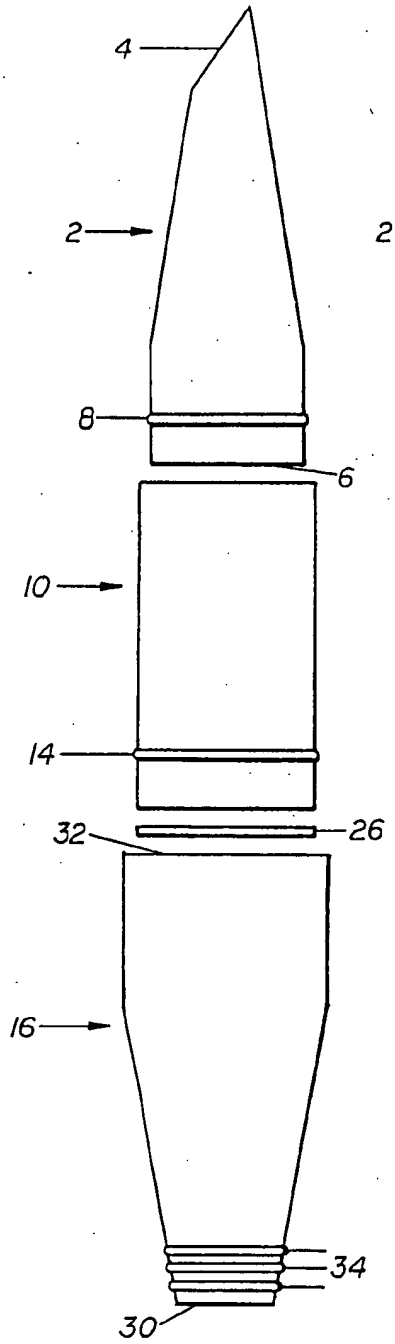


FIG. 2

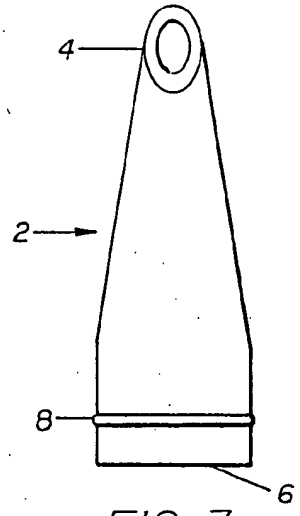
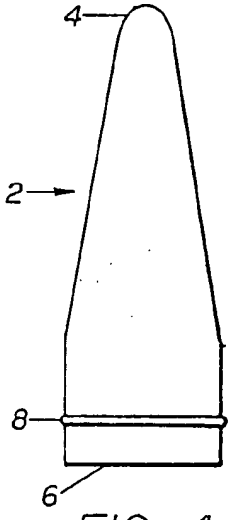


FIG. 3



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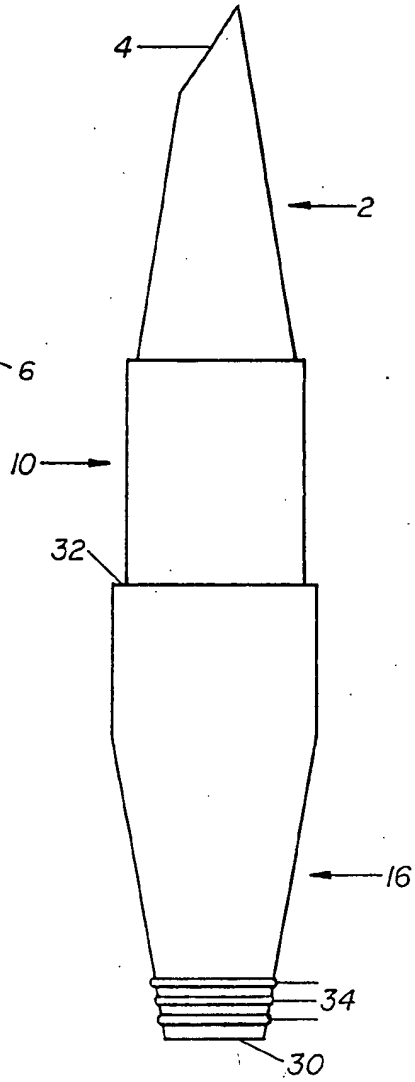

FIG. 1

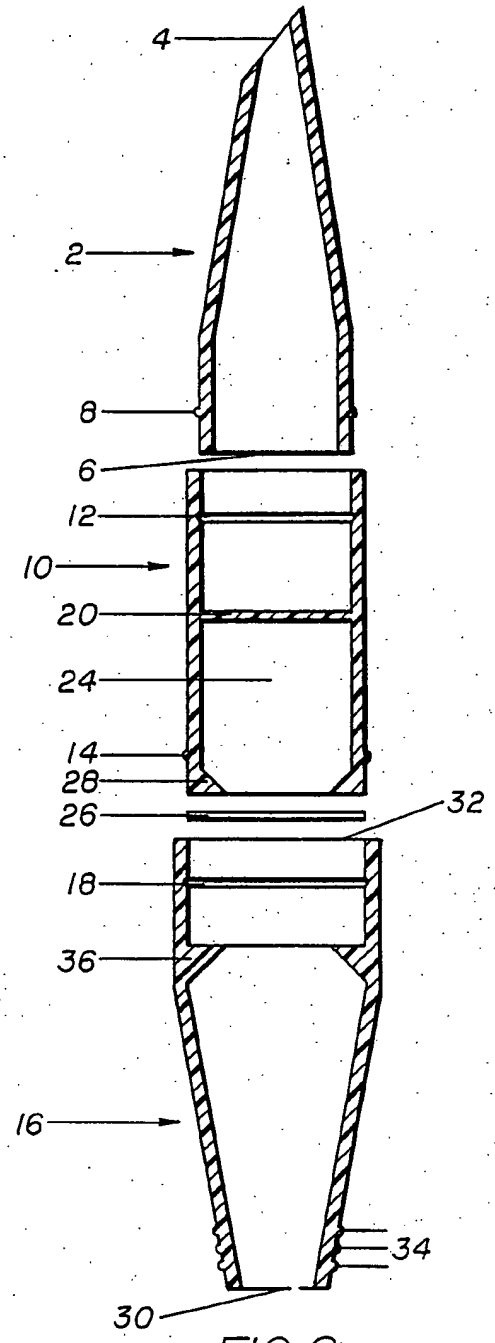
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FIG. 6

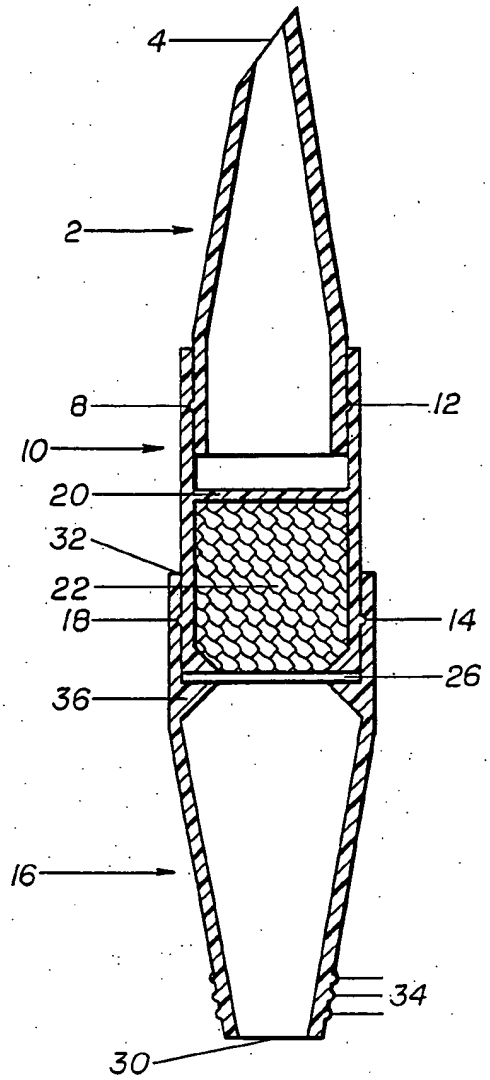
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FIG. 5

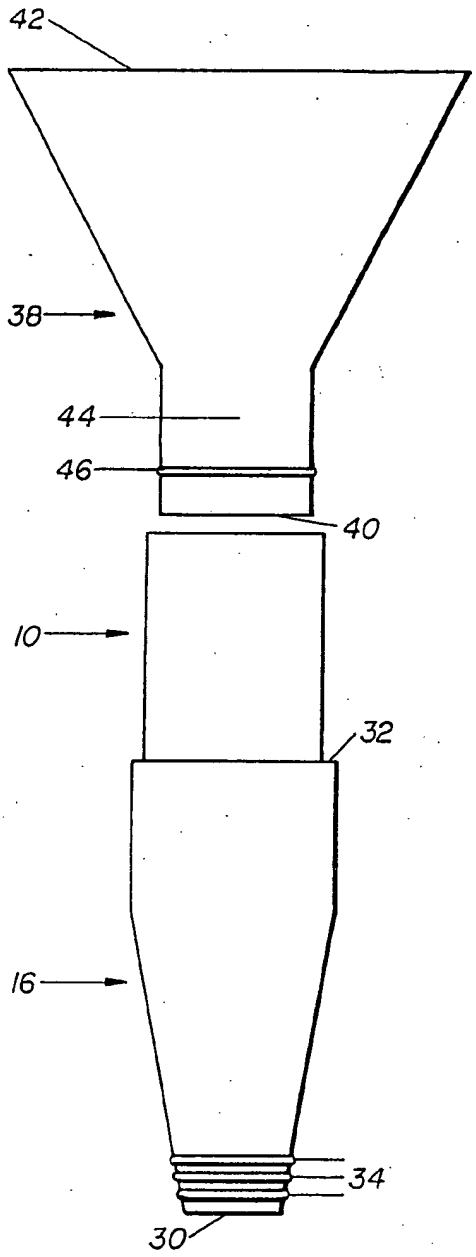

FIG. 7

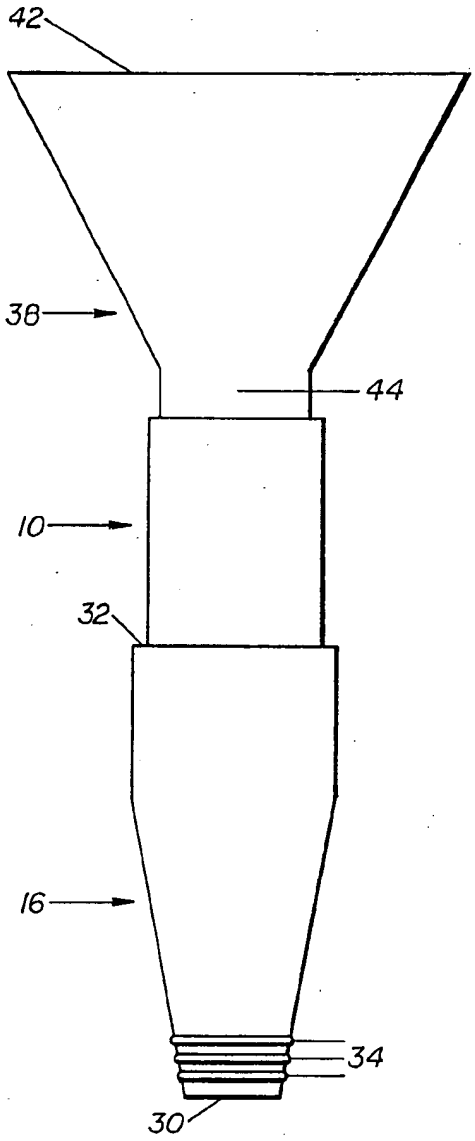

FIG. 8

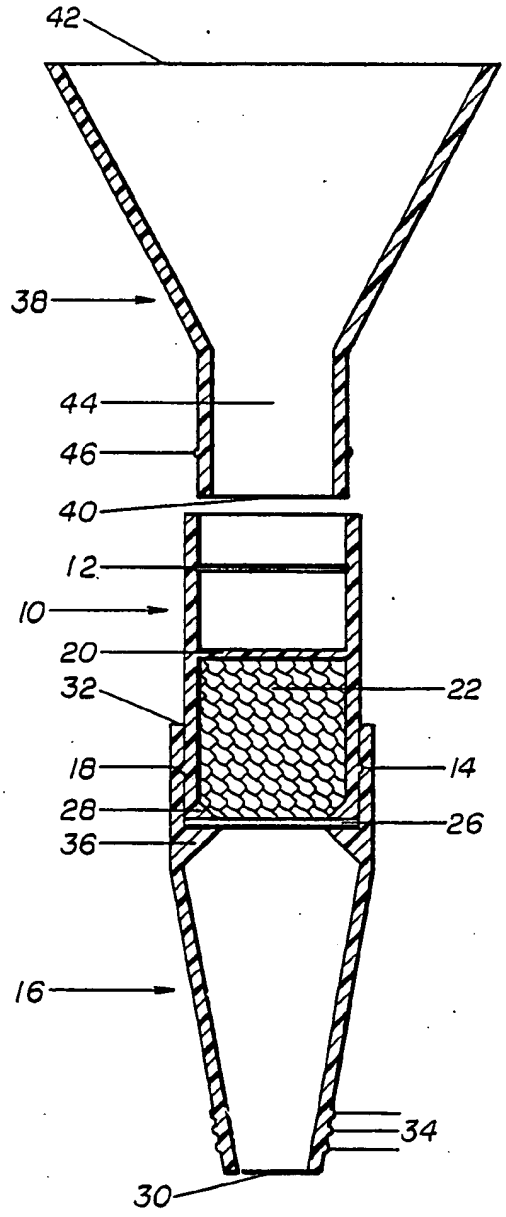
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FIG. 9

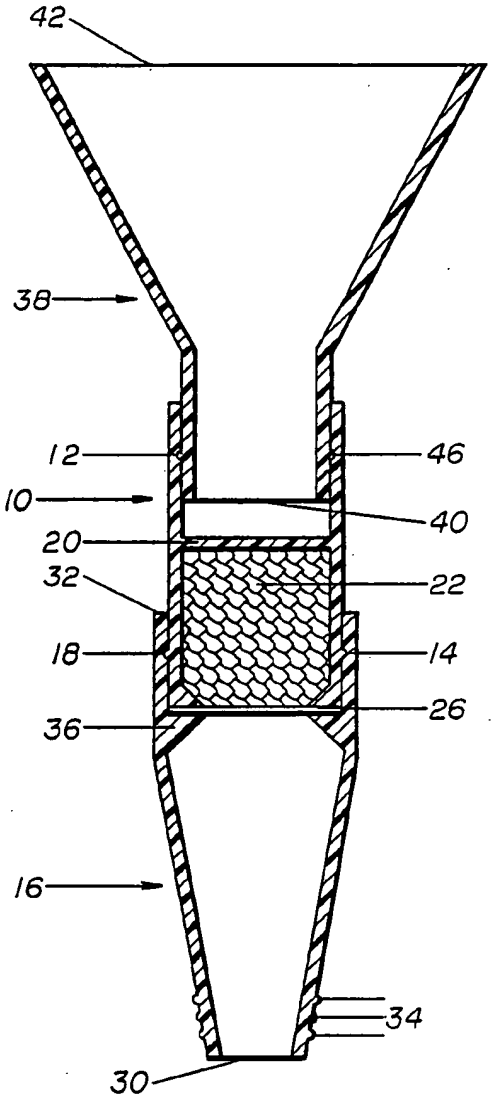
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FIG. 10

SCRAPING, COLLECTING & ELUTING APPARATUS FOR THIN LAYER CHROMATOGRAPHY AND METHOD FOR ITS USE

BACKGROUND—FIELD OF THE INVENTION

This invention relates to a device and method used in medical, chemical and biological laboratories, primarily with thin layer chromatography.

BACKGROUND—DISCUSSION OF THE PRIOR ART

Heretofore, it has been a repetitive and tiresome chore to accurately recover the adsorbent from located areas of developed thin layer chromatography plates, and then/to extract the desired substances from the adsorbent.

Small rigid metal spatulas, razor blades, and specially designed metal scraping tools are employed to remove the adsorbent from the plate, and to transfer it to a receptacle. This presents unnecessary delays and uncertainty to the scientist: (1) the metal spatula is too stiff to easily scrape the plate completely clean as it is not flexible to easily conform to the the plate during contact, hence it is time consuming and difficult to remove all of the adsorbent. (2) transfer of the detached adsorbent to a receptacle is accomplished with the potential for loss of adsorbent, a portion may be spilt unnoticed, some may remain on the spatula or on the materials used to transfer the adsorbent.

After the transfer of adsorbent to a receptacle, a means for removing the substance from the adsorbent is often necessary, elution and filtration being the most obvious and facile. Different methodologies can be used for this process: suspend the adsorbent in a suitable solvent and then pour the suspension through a filtration device, or place the adsorbent in a filtration device and pour a suitable solvent over the adsorbent. The solvent elutes the substance from the adsorbent and the eluate passes through the secondary filter. The resulting solution can be evaporated, leaving the purified substance. These methods are time consuming in preparation and clean up, particularly if the number of samples is large (each sample has to be treated independently to avoid contamination by the others), and if specific cleanup precautions are warranted while using radioactive isotopes.

Developments have led to use of vacuum assisted recovery apparatus to remove the adsorbent from the thin layer chromatography plates:

Glass pasteur pipettes were plugged with cotton or glass wool to allow air flow but not adsorbent flow through the pipette. The tapered end of the pipette was used to scrape the adsorbent from the thin layer chromatography plate while the other end of the pipette was attached to a vacuum tubing. The scraped adsorbent was aspirated into the pipette and embedded in the glass wool or cotton. The loss of adsorbent during scraping and collection was lessened. This decreased the drudgery of the scraping process although the lack of flexibility in the metal contact with the plate mentioned earlier is of equal if not greater detriment in the glass to plate contact. Additionally, the tip of the pipette would easily break if too much pressure was applied to the thin layer chromatography plate with the pipette, and the small diameter of the tip opening made scraping slow and tedious because the volume of adsorbent removed with each pass was very small.

Recovery of the substance in the adsorbent was only slightly improved. The glass wool or cotton needed to be removed from the pipette, still presenting the potential loss of adsorbent during transfer. If filtration was required, it still needed to be performed in similar fashion to that described above. Additionally, special precautions must be taken in disposal of glass especially if radioactive isotopes are used.

Improvements have led to the Chromovacs TM "Spot Recovery Device" manufactured by Spectrum Medical Industries, Inc, "Sample Recovery Tubes" manufactured by Whatman, Inc. and Wheaton Industries, "Recovery Tubes" manufactured by Alltech Associates, and the "TLC Zone Recovery Pipet" manufactured by Radnoti. These have alleviated some of the problems of inaccuracy, inconsistency, and costly time consumption, but not all. Being constructed of nonflexing glass, they are unable to easily scrape the thin layer chromatography, are breakable in shipping and usage (which can frustrate both retailer and customer), nonrepairable, and potentially injurious. All these products are vacuum assisted and aspirate the adsorbent into a chamber where it is embedded in a glass wool plug or some type of filter.

Chromovacs TM "Spot Recovery Device" is simply a cylinder tapered at both ends with a glass wool plug in the middle. It is not much of an improvement over the stuffed pasteur pipette in function as the glass wool still needs to be removed from the tube and manipulated as described above, still presenting the problem with transfer and filtering. It is not reusable and has a function limited to scraping and collecting the located area. Disposal problems are the same as with pasteur pipettes. Their cost ranges from $31.50 to $34.00 per package of 25.

Whatman, Inc.'s "Sample Recovery Tubes" have some advantages in function, but lose those advantages in cost, each tube ranging from $25.00 to $40.00. It is similar to Spectrum Medical Industries, Inc.'s Chromovacs TM with an added improvement: instead of a glass wool plug it has a permanent glass microfiber filter. They can be purchased in different sizes to accomodate different volumes of scraped adsorbent (0.5, 1.5, and 30 ml) and with a "medium" (in the 0.5 or 1.5 size) or "coarse" (in the 30 ml size) filter porosity. In addition to scraping and collection, it also can be used to filter out the adsorbent, allowing the substance to be extracted without the use of other apparatus. However, it is awkward to apply the solvent through the opening of the tube due to its small size. Furthermore, the permanent filter is of no advantage if a filtration process using a different porosity from the installed glass microfiber filter is required. One is not able to vary the filter and therefore is not able to vary the porosity of the filtration. It is marketed as reusable, but difficult to clean, also due to its small openings and the fact that it does not separate. It is expensive and unless one can afford to purchase many, the handling of numerous samples simultaneously can become even more tiresome as the tube would need to be cleaned repeatedly between samples.

The "Sample Recovery Tube" manufactured by Wheaton Industries is more complex. The collection chamber can be adjusted to accomodate different volumes of adsorbent removed. The adsorbent is aspirated into the device and trapped on a permanent frit or dis-

posable filter paper. The method of scraping, collecting and eluting the sample is similar to the method outlined for Whatman's product with the exception that the disposable filter paper can be removed before elution. The device separates into many parts and comes with a 5 ml funnel to facilitate the elution process. There are many small pieces to this device which can be easily broken or lost. It is also time consuming to clean, and expensive to replace as they cost $51.05. Their high price also makes it very costly to purchase many for simultaneous use.

The "Recovery Tube" marketed by Alltech Asssociates is virtually the same as the "Sample Recovery Tube" manufactured by Whatman, Inc. It is available in varying chamber volumes of 1, 2 and 25 ml. These are more costly than Whatman's, ranging from $45.00 to $54.00. Similarly, they are difficult to use and clean, and they offer no filtration variation.

Radnoti's "TLC Zone Recovery Pipet" is only useful for recovering small volumes of adsorbent. The adsorbent is aspirated onto a filter disc where it is trapped. After disassembling the pipet, the disc is removed and either centrifuged to dislodge the scraped adsorbent or eluted to recover the substance in the adsorbent. It, like Wheaton's "Sample Recovery Tube", separates into many parts and gaskets which can easily be lost. It too is very expensive: $54.00 each; replacement filter discs costing $21.60 for 6 ($3.60 each), and replacement gaskets costing $8.40.

Other devices employing similar methods of aspirating the adsorbent from the plate capture the adsorbent in small test tubes or embed the adsorbent in thimbles of porous plastic or other similar material. These also are expensive: a "Zone Collector" manufactured by DESAGA GmbH in Heidelberg, West Germany sells for $29.00 to $30.00 each, and the thimbles are $55.00 per lot of 25 ($2.20 each). Some of these devices require scraping with a spatula prior to their use. These methods, while making improvements, are expensive, cumbersome and still require the lengthy handling of samples to extract the desired substance from the adsorbent enmeshed in the thimble or caught in the test tube.

SUMMARY OF THE INVENTION

What is required is a facile, quick, accurate, and consistent method to remove the adsorbent from the located area on the developed thin layer chromatography plates, collect the scraped adsorbent, and extract the substance from the collected adsorbent. Ideally this would be done at a low enough cost to more than compensate for the money expended to purchase the apparatus in both time saved as well as in the increase of experimental reliability.

According several objects and advantages of the present invention meet these requirements:

The present invention is versatile: it is a scraper, collector and extractor. It completes all three functions easily in one small apparatus, eliminating the loss of adsorbent in both collection and transfer because scraping, collection, and extraction are accomplished within the same device. The device contains a filtration means that can be varied for different applications; filters of differing porosities and solvent resistances can be interchanged. It can accomodate an unlimited range of adsorbent volumes, depending upon its construction.

The present invention gives precise, reliable, consistent results and is easier and quicker to operate than other methods available. It is lightweight, compact and is made to fit comfortably in one's hand. This apparatus is easily disassembled, and reassembled, and its parts can be cleaned and replaced if a reusable market is taken. It has a broad scraping edge giving a large area of contact between the edge and the plate, allowing removal of a large volume of the adsorbent with each pass of the device over the thin layer chromatography plate. The device saves time, and the time saved increases exponentially as more samples are handled.

It can be molded of durable, inexpensive, disposable, solvent resistent, rigid but flexible polymers such as polypropylene, allowing conforming contact between the scraper and the thin layer chormatography plate. The present invention does not need to be made of glass, eliminating the possibility of breakage, injury and resulting liability. It can be manufactured, assembled and packaged easily at low cost, less than $0.12 each in large quantities, allowing a much lower cost alternative to scientists, and a large margin for profit for manufacturers. This apparatus can be marketed as disposable or reusable, either way allowing greater savings in time and money than other similar existing products, potentially capturing the market.

These objects and advantages of the invention alleviate the disadvantages of the aforementioned products. Other methods present difficulties in removing the adsorbent, collecting all of the adsorbent without loss, transfering the adsorbent without loss, and eluting the desired substance from the adsorbent without lengthy preparation, cleanup and usage of additional laboratory equipment. They are also very costly. Further advantages and objects of my invention will become apparent from a consideration of the drawings and ensuing description of it.

This invention has a surface for scraping the adsorbent off the thin layer chromatography plate and a reservoir to accomodate the adsorbent after it has been scraped from the plate. A vacuum source is connected to this device to aspirate the adsorbent into the reservoir after it has been removed from the plate. A removeable, exchangeable and therefore variable filtration means is used to retain the collected adsorbent in the invention. This device can be separated into its different parts to further facilitate it's use.

On one piece is a surface for scraping and manipulating the adsorbent. This portion attaches to another piece which accomodates the collected media. This second piece connects to a third piece which connects the whole device to a vacuum source. A gas and liquid permeable filter is located at the junction of the second and third pieces. All of these connections are airtight and use snap together fittings that are separable by hand strength and dexterity.

The reservoir is bordered on one side by the filter and on the other side by a retaining screen. This reservoir contains a filtering and retaining material such as glass wool or cotton that allows the adsorbent to easily embed itself in it, does not allow the adsorbent to easily escape. The retaining screen keeps the primary filtration material from inadvertantly being removed from the device. The filter serves to retain the adsorbent in the device during elution while allowing the solvent and desired soluble sustance (organic and inorganic compounds) to pass therethrough.

Although inexpensive, disposable polypropylene is recommended, the material from which the device is constructed may be one that will allow cleaning, autoclaving and reuse.

This device allows the functions of removal of adsorbent from the thin layer chromatography plate, collection of the removed adsorbent, and extraction of substances embeded in the adsorbent to be acomplished without additional laboratory apparatus.

This device can also be made into kit through the interchangeability of parts and the addition of a funnel. The funnel addition will allow a greater volume of liquid to flow through the device and facilitate the extraction process. This funnel is addable to the device using an attachment similar to the scraping portion mentioned earlier.

This method quickly, easily and accurately recovers located samples on developed thin layer chromatography plates with a single device by (1) detaching the adsorbent from the plate with the scraping portion, (2) aspirating the detached adsorbent into the device via attachment to a vacuum source, (3) collecting the adsorbent in a chamber located inside the device, (4) separating the device into a plurality of parts, (5) eluting the substances from the captured adsorbent, (6) filtering the resultant eluate. The resultant solution may then be collected and subjected to evaporation to recover the sample in a pure and dry form. This method can be enhanced by the addition of the funnel to allow easier addition of a solvent and therefore facilitate the elution process.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a side view showing the device assembled and ready for use;

FIG. 2 is a side view of the device disassembled showing its different parts separately;

FIG. 3 is a bottom view showing the scraping portion of the device;

FIG. 4 is a top view showing the scraping portion of the device;

FIG. 5 is a cross-sectional side view showing the device of FIG. 1 assembled and ready for use;

FIG. 6 is a cross-sectional side view showing the device of FIG. 2 disassembled to reveal its separate parts without the primary filtering material;

FIG. 7 is a side view showing the device with the scraping portion of the device removed and the funnel in position to be fastened to the device;

FIG. 8 is a side view showing the device with the scraping portion removed and the funnel attached;

FIG. 9 is a cross-sectional side view showing the device of FIG. 7 with the scraping portion removed and the funnel in position to be fastened to the device; and

FIG. 10 is a cross-sectional view showing the device of FIG. 8 with the scraping portion removed and the funnel attached.

LIST OF REFERENCE NUMERALS:

2—scraper
4—tip of scraper, scraping edge
6—base of scraper
8—raised bead on scraper
10—collector
12—recess in collector
14—raised bead on collector
16—vacuum attachment
18—recess on vacuum attachment
20—retaining screen
22—primary filtering and retaining material
24—reservoir
26—gas and liquid permeable filter
28—filter securing lip
30—tip of vacuum attachment, apex of vacuum attachment
32—base of vacuum attachment
34—series of raised beads on vacuum attachment
36—filter holding lip
38—funnel
40—base of funnel
42—top of funnel
44—neck of funnel
46—raised bead on funnel

DETAILED DESCRIPTION OF THE INVENTION

As shown in the embodiments of FIGS. 2 and 7, the device is composed of four primary parts. All parts with the exception of scraper 2 are radially symmetric.

Scraper 2 is shaped as a cone with its tip 4 sliced off at an angle aproximately 60 degrees from parallel to the base 6 of the cone. The length of scraper 2 is approximately three times the diameter of its base 6. Scraper 2 is hollow, the inside of the cone being smooth all the way from base 6 to sliced tip 4. The outside of scraper 2 is smooth from base 6 to apex 4 with the exception of a raised bead 8 around the circumference of the cone approximately ⅛ of the distance from the base 6 to the tip 4. This bead 8 is approximately ⅓ the chosen thickness for scraper 2 and will allow for an airtight "snap" together fitting with collector 10 by corresponding with a similar recess 12 around the inner surface of collector 10. With the exception of tip 4, scraper 2 is radially symmetric.

Collector 10 is cylindrically shaped. It is approximately twice as long as it is wide and about 4/5 the length of scraper 2. Collector 10 is hollow and its walls are approximately the same thickness as scraper 2. The inside diameter of collector 10 corresponds with the outside diameter of scraper 2. The outside of collector 10 is smooth along its entire length except for a raised bead 14 around the circumference of collector 10 about 4/5 of the distance along collector 10 from its end attached to scraper 2. Bead 14 is similar to raised bead 8 on scraper 2, being approximately ⅓ the chosen thickness for collector 10. Bead 14 will allow for an airtight "snap" together fitting with vacuum attachment 16 by corresponding to a similar recess 18 around the inner surface of vacuum attachment 16. Across the width of collector 10 at approximately ½ its length is a retaining screen 20. The size of the openings in screen 20 are not of great importance, needing only to be small enough to restrain primary filtering and retaining material 22 from moving out of reservoir 24 in the direction of scraper 2. Primary filtering and retaining material 22 is loosely packed in chamber 24 from retaining screen 20 to the end of collector 10 farthest from scraper 2. At this point primary filtering and retaining material 22 is bordered by a gas and liquid permeable filter 26. The inside of collector 10 is smooth along its entire length with the exceptions of recess 12, retaining screen 20 and a filter securing lip 28. Filter securing lip 28 gradually decreases the inside diameter of collector 10 at the end of collector 10 farthest from scraper 2. Filter securing lip 28 provides a greater area against which gas and liquid permeable filter 26 can be secured. Lip 28 begins approximately 1/5 of the length of collector 10 from vacuum attachment 16 and gradually increases the thickness of the walls of collector 10 in the direction of vac-

uum attachment 16 to approximately 2 ½ times their thickness elsewhere.

Vacuum attachment 16 is conically shaped similar to scraper 2 with its apex 30 sliced off not at an angle but parallel with its base 32. Vacuum attachment 16 is approximately 2 1/3 as long as its width. It is hollow, and the thickness of the walls of vacuum attachment 16 is similar to the thickness of the walls of scraper 2 and collector 10. The inside diameter of vacuum attachment 16 corresponds with the outside diameter of collector 10. The outside surface of vacuum attachment 16 is smooth from base 32 to apex 30 with the exceptions of a series of raised beads 34 around the circumference of vacuum attachment 16 to allow for a more secure and airtight attachment with a vacuum hose to which vacuum attachment 16 will be joined during operation. Series of raised beads 34 begin approximately 1/24 of the length of vacuum attachment 16 from the end of vacuum attachment farthest from collector 10, and continue for about ⅛ of the length of vacuum attachment 16 towards collector 10. Series of raised beads 34 are approximately ¼ to ½ the chosen thickness of vacuum attachment 16. The inside of vacuum attachment 16 is smooth from base 32 to apex 30 with the exceptions of recess 18, and filter holding lip 36. At a distance of approximately ¼ of the length of vacuum attachment 16 from base 32, filter holding lip 36 abruptly decreases the diameter and then gradually increases the diameter of the inside surface of vacuum attachment 16. The thickness of the walls of vacuum attachment 16 increase to approximately 3 ½ times their thickness elsewhere. The thickness gradually diminishes towards its original thickness approximately 4/13 of the length of vacuum attachment 16 from base 32.

Gas and liquid permeable filter 26 is disc shaped and the same diameter as the outside diameter of collector 10. Its thickness is not of great importance except that it needs to fit tightly and securely between filter securing lip 28 and filter holding lip 36, allowing air and liquid flow only through the filter 26 and not around the edges of the filter 26.

Funnel 38 is approximately three times as high as it is wide at base 40, and the same height as its width at top of funnel 42. The base of the funnel 40 has the same dimensions as the base of scraper 2 allowing for interchangeability with scraper 2 in attachment with collector 10. The width of the neck of the funnel 44 is constant for about ⅓ its height, where it flares out of the top of the funnel 42. The inside of funnel 38 is hollow and the surface is smooth from base 40 to top of funnel 42. Funnel 38 is of similar thickness as scraper 2, collector 10 and vacuum attachment 16. The outside surface of funnel 38 is smooth from base to apex with the exception of a raised bead 46 approximately 1/10 the the height of funnel 38 from base 40. Bead 46 has the same dimensions as raised bead 8, approximately ½ the chosen thickness of funnel 38 and allows for an airtight "snap" together fitting with collector 10 via the corresponding recess 12 on collector 10.

All four pieces, scraper 2, collector 20, vacuum attachment 16, and funnel 38 can all be manufactured in single pieces via injection molding. The retaining screen 20 can be molded in collector 10. The filter 26 can be specially manufactured, or the device can be manufactured to accomodate preexisting filters. Glass wool, cotton or other suitable primary filtration material is readily available.

While the above description contains many specifications, these are not to be construed as limitations on the scope of the invention, but rather as exemplifications of one prefered embodiment thereof. Other possible variations can be envisioned, but remain within the scope of my invention. A non-exhaustive treatment of the variations are listed below.

The connection 14 & 18 between collector 10 and vacuum attachment 16 can be one that will not allow disassembly once it is snapped together. This would be an attractive option if the device was marketed to be disposed of after use.

Collector 10 and vacuum attachment 16 can alternately be molded in one piece, inseparable at the position where the filter 26 is in the drawings. This amalgamation of collector 10 and vacuum attachment 16 may be molded with a slight indentation corresponding to the circumference of the disc filter 26 to ensure a tight fit between the filter 26 and the collector/vacuum attachment piece. After the primary filtering material 22 is inserted the retaining screen 20 can be wedged into the collector/vacuum attachment, or a recess corresponding to the circumference and position of the screen 20 can be molded into the collector/vacuum attachment as was done to accomodate the filter 26. The disadvantages are that the filter 26 is not easily exchanged and it will be difficult to ensure a tight fitting between the collector/vacuum attachment and the filter 26. This assembly does not afford the security of all parts as in the recommended means of manufacturing.

Alternately a porous plastic filter can be molded to correspond with the insided dimensions of the middle half of the collector/vacuum attachment (from about ¼ to ¾ of its length). This depth filter will perform the same function as the primary filtering material 22 and the disc filter 26, capturing the adsorbent in its pores and serving as a filter during elution. The porous plastic filter can be positioned by wedging it into the collector/vacuum attachment and employing a stiff retaining screen (with corresponding recessions molded in the collector/vacuum attachment to ensure tightness of fit) to secure it on the side of the filter near the base of the collector/vacuum attachment. The porous plastic filter could then be wedged into the collector/vacuum attachment, the protrusions preventing the filter from accidentially removing itself from the collector/-vacuum attachment. This would alleviate the need for the retaining screen. Other means may be employed to secure the porous plastic filter. This method of construction is not recommended as porous plastic filters are more expensive and provide no low cost filter variation.

This invention is recommended to be made of polypropylene or other suitable moldable polymer, but it is not restricted to such material. If a glass apparatus is needed for compatibility with a given solvent, the funnel 38, collector 10 and vacuum attachment 16 or the collector/vacuum attachment can be manufactured of glass, and scraper 2 of any given material as it will not be exposed to the solvent during elution. If made of polypropylene, glass or other suitable polymer, this invention is durable enough to be washed and reused, even autoclaved if necessary.

This invention can be used with just the primary filtering material 22 or with just the secondary disc filter 26 in any of its embodiments. However, with only the secondary disc filter 26, collection will be jeopardized as there will be no material in which the adsorbent

can embed itself, hence the potential for adsorbent loss is great. Solely using the primary filtering material affords a measure of filtration, adequate for some procedures, but allows no variation in filtration porosity.

The primary filtering material **22** may be of cotton, glass wool or any other suitable material. The retaining screen **20** is not necessary in any of the embodiments but recommended to ensure the retention of the primary filtering material **22**.

The secondary gas and liquid permeable disc filter **26** can be made of any suitable material: glass microfibers, cotton, acrylic copolymers, etc. A backing can be added as a filtering surface in any of the embodiments if more strength is required than that provided by the secondary filter alone.

The overall size of the device can be varied, specifically to accomodate differing volumes of adsorbent being scraped. Collector **10** or the collector/vacuum attachment piece can also be deliberately sized to accomodate preexisting funnels which provide a good seal instead of specifically manufactured funnels.

The snap together fittings **8** & **12**, **14** & **18**, **12** & **46** in all the embodiments may be replaced with screw together or other suitable fittings.

The color of the scraper can be varied and a company name and logo can be imprinted on the device.

The shapes and embodiments of this invention are not restricted to these in the drawings and can be altered to accomodate different manufacturing and molding methods and different parts used.

This does not constitute an exhaustive treatment of the many and varied ways this invention can be constructed. Accordingly, the scope of this invention is determined not by the embodiments illustrated via drawings and text, but rather by the appended claims and their legal equivalent.

OPERATION OF THE INVENTION

In order to use this invention, it must be properly assembled as shown in FIGS. **1** and **5**, and attached at the base of vacuum attachment **16** to a vacuum source via a flexible hose (not shown). After developing the thin layer chromatography plate and locating the areas to be collected, the slanted edge of scraper **2** is used to remove the adsorbent from the chromatography plate. The adsorbent will be aspirated up through scraper **2** into reservoir **24** where it is embedded in primary filtering material **22**. The thin layer chromatography plate can easily be scraped clean with the contact the scraping edge **4** provides with the plate. Stray particles of adsorbent can be aspirated into the primary filtering material to ensure that no adsorbent is lost.

The user then gently and slowly separates scraper **2** from the rest of the device, keeping base **30** pointed towards the ground. Although the adsorbent is embedded in the primary filtering and retaining material **22**, jerky movements should be avoided to ensure retention of all collected adsorbent. Attach funnel **38** to collector **10** in the same fashion that scraper **2** was connected. Hold the apparatus over a desired receptacle with the base **30** pointed into the receptacle. Aliquot a suitable solvent through the opening of the funnel **42**. As the solvent passes through primary filtering material **22**, it will draw the substance out of the absorbent and carry the substance and some of the adsorbent with it. As the eluate and the suspension of adsorbent arrives at the gas and liquid permeable filter **26**, the adsorbent will remain in reservoir **24**, but the solvent containing the substance will pass through the gas and liquid permeable filter **26** into the desired receptacle. Additional aliquots may be necessary to ensure complete elution of the substance. When the elution/filtration process is finished, the eluate can then be subjected to an evaporative technique; as the solvent evaporates, one is left with the pure recovered substance.

Filters **26** can be varied depending on the desired purity of the recovered substance, adsorbent particle size and the desired speed of filtration (larger porosity filters allow quicker filtration). To exchange filters **26**, primary filtering material **22**, or clean, the device can be separated into its component parts as shown in FIGS. **2** and **6**. Primary filtering material **22** can be inserted into reservoir **24**, and filter **26** can be placed against filter holding lip **32** inside vacuum attachment **16**. With base **30** pointed toward the ground, the filter **26** will be held in place by the walls of vacuum attachment **16** as collector **10** is snapped together with vacuum attachment **16**.

When handling many samples, a number of scrapers can be used simultaneously to save time. As each sample requires a different scraper, each can be separated into their respective parts and the elution process can be done on all the samples simultaneously. No time is lost while waiting for the solvent to pass through the primary filtering and retaining material and secondary filter.

If collection of the adsorbent is all that is required the primary filtering material can be removed to allow easy recovery of the adsorbent.

CONCLUSION AND SCOPE OF THE INVENTION

This device was invented to eliminate the time consuming, cumbersome and inaccurate means of manipulating developed samples on thin layer chromatography plates. The reader will see that this device provides the scientist with the following advantages: it facilitates the process, saves time and improves experimental reliability.

The invention will allow for the facile removal of the adsorbent from the plate, will ensure the retention of virtually all the adsorbent during collection, and will allow for the facile elution and purification of the sample. This is accomplished with normal use of laboratory equipment, minimizing time spent in preparation and cleanup to allow maximum use of time. All this can be accomplished at low cost to the scientist.

The use of this invention is not limited to thin layer chromatographic applications. Any time a substance needs to be removed collected and eluted to recover a soluble element contained in said substance, this invention can be considered for use.

I claim:

1. A device for the removal and collection of anhydrous particulate media, and for the extraction of substances from said media, comprising:

(a) a rigid but flexing surface for manipulating said media;

(b) a reservoir located inside said device of sufficient size to accomodate the volume of collected media;

(c) a filtration means for retaining said media in said reservoir during extraction of said substance from said media; and

(d) connection means for connecting said device to a vacuum source wherein said vacuum causes the removed media to pass into said reservoir, said device being separable into a plurality of parts.

2. The device of claim 1, wherein said filtration means includes a removable, exchangeable and therefore variable filter.

3. The device of claim 1, wherein

(a) said rigid but flexing surface is located on a first piece which is attached to a second piece having said reservoir located therein;

(b) said connection means is located on a third piece which is attached to said second piece; and

(c) said filtration means is located in said device in a fashion whereby all solids, liquids and gasses must pass through the filtration means to pass through the device.

4. The device of claim 1, wherein said reservoir for the collection of media is located posteriorly with respect to the direction of gas flow to a retaining screen, and anteriorly with respect to the direction of gas flow to a removable, exchangeable and therefore variable filter.

5. The device of claim 1, wherein said reservoir contains a material in which said media is easily embedded but removed only with significantly more effort than necessary to embed said media in said material.

6. The device of claim 1, wherein said device is constructed of such material to allow intentional and economical disposal after use.

7. The device of claim 1, wherein the components of said device are easily disassembled and reassembled with common hand strength and dexterity.

8. The device of claim 1, wherein the functions of removal, collection and extraction are accomplished within said device whereby;

(a) probability of loss of said media containing said substance is not increased due to transfer of said media; and

(b) said functions are accomplished without additional laboratory apparatus.

9. The device of claim 1, further comprising a funnel addable to said device whereby a larger volume of solvent is added to a larger area whereby the said extraction of said substances from said media is facilitated.

10. A kit for removing anhydrous particulate media from a surface, for collecting said media, and for subjecting the collected media to elution and filtration whereby substances embedded in said media are recovered with greater ease, less time, cost and equipment than present methods comprising:

(a) a rigid but flexible edge for scraping and removing media attached to a surface;

(b) a collection and retention means adjacent to said rigid but flexible edge for collecting and retaining the volume of removed media;

(c) a filtration means for separating said media from said substances; and

(d) an attachment means adjacent to said collection means whereby said kit is attached to a vacuum source.

11. The kit of claim 10, further comprising a funnel having a larger opening than the piece to which it attaches, addable or interchangeable with another piece in said kit whereby the addition of larger volumes of solvents are added to a greater area to facilitate the processes of elution and filtration.

12. The kit in claim 10, having a plurality of pieces, comprising:

(a) a first piece having said rigid but flexible edge for removing said media when adhered to a surface;

(b) a second piece connected with said first piece having said collection and retention means therein for collecting and retaining the removed media;

(c) a third piece connected to said second piece having said attachment means for connection to a vacuum source; and

(d) a fourth piece interchangeable with said first piece for connection to said second piece, said fourth piece having a larger diameter opening than the diameter of said second piece.

13. The kit in claim 10, wherein said filtration means is removable and exchangeable, and therefore variable.

14. The kit in claim 10, wherein some pieces are constructed of polypropylene and intended to be disposed of after use.

15. A method using a single device for recovering substances from anhydrous particulate media, comprising the steps of:

(a) detaching said media from a surface with a rigid but flexible scraping edge on said device;

(b) aspirating the detached media from said surface via collection of said device to a vacuum source;

(c) collecting the aspirated media in a chamber located in said device;

(d) separating said device to provide a larger opening through which a liquid can be introduced into said device;

(e) aliquoting a solvent over said media remaining in said device, whereby said substances are eluted from said aspirated media; and

(f) filtering the resultant eluate through a filtration means located in said device, whereby said anhydrous particulate media is separated from said eluate and retained in said device.

16. The method in claim 15, further comprising the steps of:

(a) collecting media free eluate in a receptacle;

(b) evaporating said solvent from said eluate to recover said substance in a dry pure form.

17. The method in claim 15, further comprising the attachment in a manner impermeable to solids, liquids and gasses of a funnel to the separated device to allow an even larger opening through which a liquid may be introduced into said device and to allow a greater volume of said liquid to be introduced during a given aliquot of said liquid.

18. The method in claim 15, further comprising having a means for retaining said media in said chamber irrespective of position of said device with respect to gravity or detachment of said device from said vacuum source.

19. The method in claim 15, further comprising the pre-intentional disposal of some (or all) of said device after use.

20. The method in claim 15, further comprising removing, exchanging and therefore varying said filtration means when differing porosity filtration is required.

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[54] EXTRACTION VIAL

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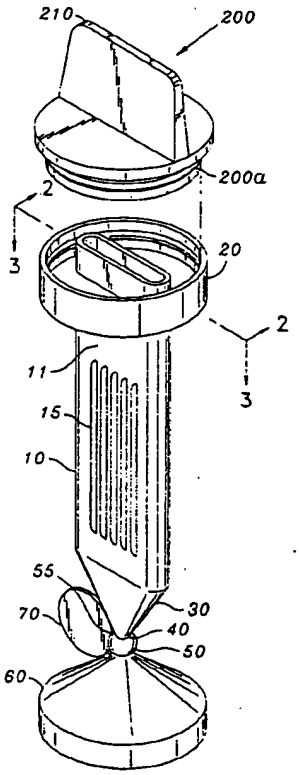
Attorney, Agent, or Firm—William H. May; Gary T. Hampson

[57] ABSTRACT

An extraction vial includes a reservoir portion having generally parallel walls, a neck portion defining an opening into the reservoir portion and a channel surrounding the opening, a nozzle portion integral with reservoir portion at a second end of the reservoir, a sealing closure that closes the nozzle, a base integral with the sealing closure and including a support surface adapted to support the vial in an upright position, and a cap received and removably maintained within the channel. An extraction liquid may be contained within the vial for extracting sample from a carrier. The vial may be used by adding the carrier to the extraction liquid within the vial, closing the vial with the cap, agitating the vial to release sample from the carrier, and removing at least the sealing closure or, preferably, the sealing closure and base whereby extraction liquid can be expelled from the vial.

4 Claims, 1 Drawing Sheet



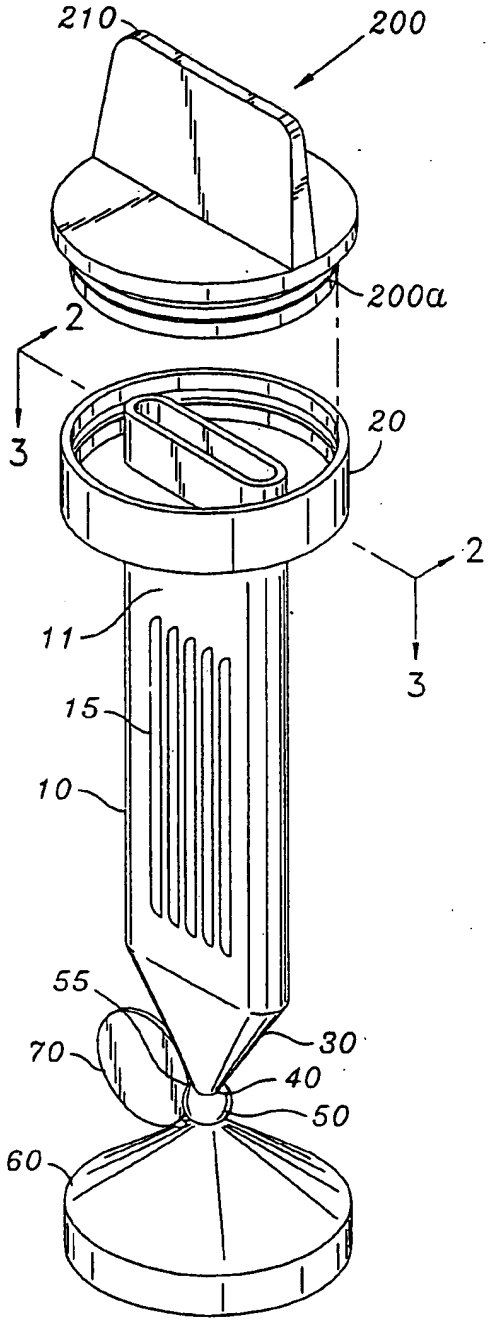


FIG. 1

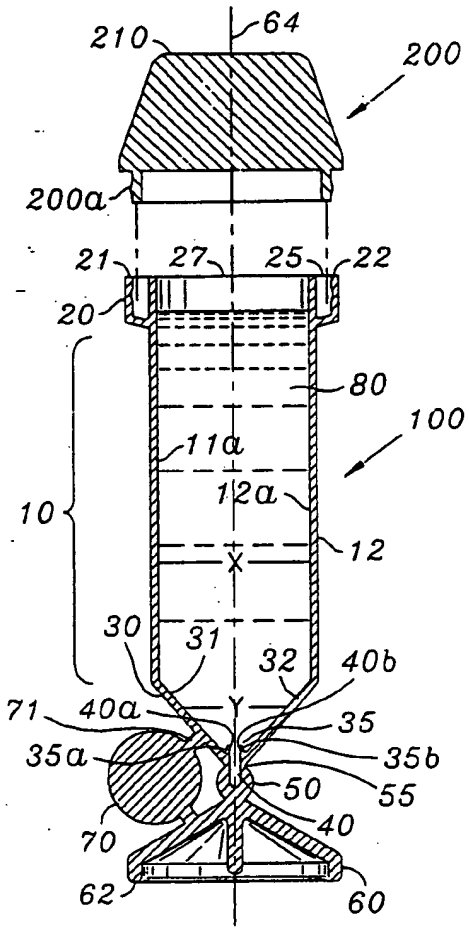


FIG. 2

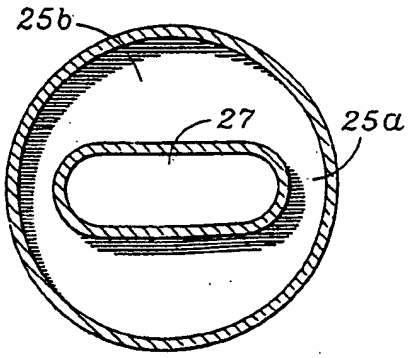


FIG. 3

EXTRACTION VIAL

FIELD OF THE INVENTION

The present invention is directed to immunochemical testing in general, and in particular to extraction vials and kits comprising an extraction solution for use in extracting an analyte from a test sample for deposit of the analyte onto a testing device.

BACKGROUND OF THE INVENTION

Diagnostic test matrices have revolutionized the health care industry by focusing on relatively easy-to-use devices. For examples, pregnancy-testing dip sticks are commonplace in market facilities throughout the world. Fecal occult monitoring devices, such as the Hemoccult ® brand fecal occult blood test, allow for obtaining samples in the privacy of the patient's home whereby analysis of the sample can be accomplished at a screening facility.

Another form of such test matrices are chromatographic-type assaying systems. Chromatographic-type assaying systems have enjoyed widespread use in the diagnostic fields for several years. Such devices typically rely upon a format whereby a solution comprising a carrier solvent and a test sample suspected of containing an analyte to be detected, is applied to a thin, flat, absorbent medium, which typically has incorporated thereon a binding partner to the analyte. The solution is applied to the absorbent medium and moves along the medium by way of capillary action. A labeling scheme is then utilized to determine the presence of the immobilized analyte. This type of assay is generally referred to as an "immunochromatographic assay". There are two types of immunochromatographic assay types, generally referred to as "sandwich", or "capture", immunochromatographic assays, and "competitive" immunochromatographic assays.

Sandwich immunochromatographic assays typically involve mixing a sample containing an analyte of interest with either a monoclonal or polyclonal "capture" antibody to the analyte (alternatively, the sample can be added directly to a chromatographic medium having affixed thereto the capture antibody). The antibody can be conjugated to some form of label, for example, colored latex beads, chemiluminescent, enzymatic, fluorescent, radioactive, colloidal gold, etc. Thereafter, an analyte-labeled antibody complex is formed. This complex is then applied to the chromatographic medium which has a second monoclonal antibody or additional polyclonal antibodies immobilized thereon. As the complex moves along the medium, the analyte-labeled antibody complex becomes bound to the immobilized antibody, forming an immobilized antibody-analyte-labeled antibody complex. The label can then be read to provide an indication of the presence (and quantity) of the analyte in the sample medium.

Competitive immunochromatographic assays typically involve mixing a sample containing an analyte of interest with a known quantity of the same analyte having a label conjugated thereto. This mixture is then added to a known quantity of immobilized antibody to the analyte. As the mixture moves along the medium, a competition is created between the sample-analyte and the labeled-analyte: the more sample-analyte available for binding to the immobilized antibody, the less label that will be detected. Thus, the amount of sample-analyte is inversely proportional to the amount of label obtained.

Immunochromatographic assays are not without drawbacks. For example, with respect to, e.g., fecal samples, mucous-based samples, throat or vaginal swabs, etc., it is very difficult to apply these materials to the chromatographic medium directly. Thus, these materials are usually applied to the chromatographic medium, whereupon a solvent solution is added thereto, the solvent being capable of carrying the analyte of interest to a particular location on the chromatographic medium. Problems arise using this approach because the materials can clog the pores of the chromatographic medium, making chromatographic analysis extremely difficult because of interference with the flow of the solvent caused by such clogging. Additionally, these materials can include therein extraneous matter which can interfere with the analysis due to, e.g., non-specific binding with the analyte antibodies, or interference with the particular label utilized for the analyses.

An alternative to this approach is to utilize extraction or pretreatment reactions whereby the sample material is added to a liquid extracting medium such that the sample is brought from its solid or semi-solid form to a liquid form. Conventionally, this is accomplished by a technician in a laboratory setting and is carried out in small transfer vessels, whereby the extraction medium containing the sample is added to the chromatographic medium via, e.g., a pipette. This scenario raises additional problems, particularly in the areas of contamination and waste disposal. Such problems are particularly relevant with respect to the possibility of transfer of communicable diseases to the technician(s), as well as the potential for cross-contamination of samples.

What is needed, in view of the foregoing, is a device that will readily facilitate the extraction of an analyte from a sample for analysis of material in the sample, and that ensures that contamination and waste byproducts are substantially minimized.

SUMMARY OF THE INVENTION

The present invention satisfies these needs. In accordance with the invention disclosed herein, a disposable extraction vial capable of containing or alternatively, containing liquid is provided. The extraction vial is adapted for receiving a diagnostic sample carrier such that removal of the sample from the carrier is facilitated by the interaction of the sample with the extraction liquid. The extraction vial is further adapted for controlled release of a desired amount of the extraction liquid comprising the diagnostic sample onto a diagnostic test platform. Beneficially, the extraction vial containing the sample carrier and extraction liquid remaining therein can be conveniently disposed as a single unit. Test kits comprising an extraction vial and a separately contained extraction medium for addition to the vial, are further disclosed.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are presented for the purpose of reference in conjunction with the Detailed Description of Preferred Embodiments of the Invention.

FIG. 1 is a perspective view of an embodiment of the disclosed extraction vial;

FIG. 2 is a cross-sectional view taken through line 2—2 of FIG. 1; and

FIG. 3 is a cross-sectional view taken through line 3—3 of FIG. 1.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

With the exception of certain clinical samples, for example, whole blood, serum, plasma, urine, cerebro spinal fluid, other clinical samples typically require a collection step and a liquid modification step before the sample can be analyzed via diagnostic assay procedures. (The aforementioned clinical samples can, however, be utilized in conjunction with the invention disclosed herein.) As used herein "other clinical samples" include solid-form or semi-solid form materials, for example, fecal materials, epidermal scrapes, mucous, throat, vaginal swabs, etc. As used herein "liquid modification step" is intended to mean steps taken to allow for a chromatographic-type flow of such other clinical samples along a diagnostic test platform; typically, this is manifested by totally or partially liquefying the solid-form or semi-solid form test sample so that analytes of interest interspersed therein can be examined via the diagnostic test protocols.

For ease of discussion and without the intention of limiting the types of clinical samples which can be utilized in conjunction with the disclosed extraction vial, the following discussion will focus on fecal materials as an example of an applicable clinical sample, and immunochromatographic assaying procedures. It is to be understood, however, that the disclosed extraction vial can be utilized with a myriad of diagnostic assaying procedures. I.e., the disclosed vial can be utilized in conjunction with diagnostic matrices such as the Hemoccult ® brand fecal occult blood test (SmithKline Diagnostics, Inc., San Jose, Calif., U.S.A.) whereby a fecal sample is extracted from a sample carrier into the extraction vial, and the extraction liquid, comprising the sample, is applied to, e.g., a guaiac-based assay device; developing solution can thereafter be applied to the device. Those in the art are credited with recognizing the advantages to be derived from utilizing the disclosed extraction vial in related diagnostic assaying protocols.

As is well known in the art, the presence of hemoglobin in fecal samples may be indicative of colon cancer; accordingly, screening of fecal samples for the presence of hemoglobin is routinely practiced. However, as is also well known, with respect to guaiac-based fecal occult screening devices, certain foods, e.g., red meats, vegetables, etc., can give false positive results. This is because blood present in meat, or peroxidases present in vegetables, may be present in the fecal sample, and these materials may indicate, erroneously, the presence of patient-derived hemoglobin in the fecal sample.

The advent of immunochemical testing protocols has helped to improve such screening. For example, antibodies specific for, e.g., human hemoglobin, can be utilized for the detection of human hemoglobin in fecal samples. Beneficially, diagnostic test devices can fully exploit the binding specificity of such antibodies. However, because of the chromatographic aspects of such devices, it is necessary for a liquid to be added to the device, typically at a region where antibodies specific to a desired analyte are located. For example, in a "dipstick" type diagnostic device, a portion of the device is "dipped" into a liquid sample or a liquid comprising a sample. A common form of this device is a pregnancy dipstick whereby antibodies specific for human chorionic gonadotropin ("HCG", indicative of pregnancy) are coated onto a portion of the dipstick; the dipstick is inserted into patient urine. Instantly, the chromatographic aspect of such a device draws the liquid sample up through the device, whereby interactions between patient HCG, HCG-specific antibodies and some form of labeled agent (typically a labeled antibody specific for an epitope on HCG other than the first antibody epitope) indicate the presence or absence of HCG.

With solid and semi-solid samples such as fecal samples, it is necessary to extract the analyte of interest. I.e., either a liquid can be added to such a sample which has been placed directly onto a diagnostic test device (as is the case with, e.g, Hemoccult ® brand fecal occult blood monitoring devices), or the sample can be added to a liquid which, in turn, is added to the test device. From a practical perspective, the latter protocol is utilized in that in order to liquify sample added directly to a device, sufficient liquid must be added thereto; this can often lead to (relative) excessive amounts of such a liquid such that the sample can be washed from the testing device, or contamination of the instrument used to add the liquid to the sample (e.g., a pipette tip coming into contact with the sample) can occur. Focusing on a typical method of practicing the former protocol, the incidence of cross-contaminations and waste disposal is increased. This is because sample carriers (i.e., some form of material to which the sample is applied) are added to, e.g., microtiter wells, and a liquid is added to the wells. Thereafter, liquid comprising the sample is removed from the wells and added to the diagnostic test device. Thus, it is possible (due to liquid splashing, etc.) to cross contaminate the wells. Additionally, the wells must be either disposed or thoroughly washed and cleaned after use.

These problems are avoided by use of the disclosed invention which provides a container comprising a body portion defining a chamber containing an extraction fluid, the body portion defining an opening for external access into the chamber and having a predefined line of weakness in the wall portion, whereby the line of weakness in the wall portion is broken to allow for the release of the solution, preferably without the need to utilize a cutting tool. In a particularly preferred embodiment of the invention, a disposable extraction vial is disclosed, the vial comprising an elongated hollow body comprising a reservoir portion constituted by a chamber bounded by a pair of spaced, flat, generally parallel, compressible walls, said chamber comprising an extraction liquid for expulsion upon compression of the walls; a neck portion integral with an area near an end of the reservoir portion, the neck portion comprising a channel for receiving a removable cap, the channel defined by the terminal end of the reservoir portion and the neck portion; a generally cylindrical nozzle portion integral with the end of the reservoir portion opposite the neck portion, the nozzle portion comprising a passage connecting with the chamber and a leading end, the leading end of the nozzle portion being angularly offset with respect to the longitudinal extent of the chamber; a sealing closure integral with and terminating the leading end of the nozzle portion and separated therefrom by a line of weakness, the connection between the sealing closure and the leading end being such that when sealing closure is broken, the free end of the nozzle portion presents a discharge opening; a support tab integral with, extending between and connecting said nozzle portion to the sealing closure to protect the nozzle portion against inadvertent disruption; and an extraction liquid adapted for use in diagnostic analy-

sis of biological materials, the liquid located within reservoir portion.

Beneficially, the disclosed extraction vial avoids the problems noted above without compromising the testing procedure. By utilizing an extraction vial comprising an extraction liquid, a sample carrier (or a section thereof) is added directly to the vial whereby the sample material can be extracted from the carrier into the liquid. At an appropriate time, the liquid comprising the sample can be added to the diagnostic test device; thereafter, the vial containing the liquid and the sample carrier can be efficiently disposed. Advantageously, because the extraction liquid is located within the vial and the sample carrier is added thereto, problems and concerns associated with, e.g., cross-contamination, are avoided.

With reference to the figures and a particularly preferred embodiment of the extraction vial, the disposable extraction vial **100** comprises an elongated hollow body comprising a reservoir portion **10**; a neck portion **20** integral with the reservoir portion; a nozzle portion **30** integral with the reservoir portion located opposite to the neck portion; a sealing closure **40** integral with and terminating the leading end **35** of the nozzle; a support tab or mechanism **70** integral with, extending between and connecting the nozzle portion to the sealing closure; and an extraction liquid **80** include within the reservoir portion.

Extraction vial **100** is preferably composed of a chemically inert material conducive to use in conjunction with biological and chemical samples. Exemplary materials include, but are not limited to, thermoplastics such as polypropylene, polyethylene, polynitrile, polyvinyl acetate, polyethylene terephthalate, polyethylene-vinyl acetate, polyethylene-vinyl alcohol, and polyvinylchloride. Preferably, the material is capable of being subjected to injection-mold procedures, relatively inexpensive, and somewhat compressible, particularly in the area of reservoir portion **10**. Most preferably, the material is polyethylene.

Reservoir portion **10** is constituted by a chamber bounded by a pair of spaced, flat, generally parallel walls, **11** and **12**. Preferably, walls **11** and **12** are of sufficient thickness that they are both compressible. By "compressible" is meant that when external pressure is applied thereto, walls **11** and **12** are capable of slightly moving in the direction of the applied pressure and are capable of resuming their approximate original position after release of pressure. For example, when compressed by the applied pressure of a finger and a thumb. Such pressure is preferably applied after a sample carrier (not shown) has been added to the vial. Beneficially, the application and release of such pressure (i.e., "agitation") aids in the release of the sample from the carrier into the extraction liquid. In this regard, the portion of walls **11** and **12** bounding the area between the walls (i.e., portions **11***a* and **12***a* of walls **11** and **12**, respectively) are preferably substantially non-parallel with each other. By "substantially non-parallel" is meant that these portions of the walls further comprise features that are out of the general plane of a straight line defined by walls **11** and **12**. For example, a series of ridges (not shown) can be located along walls **11***a* and **12***a*; alternatively, deformations (i.e., indentations, protrusions, etc.) can be incorporated along walls **11***a* and/or **12***a*. The function of such ridges is to assist in agitation of the extraction liquid and the sample carrier. I.e., as walls **11** and **12** are compressed, the increased surface area of walls **11***a* and **12***a* occasioned by such ridges increases the agitational movement of the liquid, thus increasing the likelihood of entry of additional sample material into the liquid.

Preferably, walls **11** and **12** further include "grip assisters" **15** (shown with respect to wall **11** in FIG. **1**), e.g., a series of ridges which allow the user to more readily maintain a firm grip on device **100** for purposes of, e.g., compression of walls **11** and **12** for agitation of the liquid and/or release of the liquid onto an diagnostic test platform. As should be apparent, such grip assisters can be of any shape; the function thereof is to assist in the handling of device **100**.

Neck portion **20** is integral with reservoir portion **10**. Most preferably, neck portion **20** comprises a channel **25** for receiving a removable cap **200**; channel **25** is defined by walls **11** and **12** and portions **21** and **22** of neck **20**. Referencing FIG. **3**, region **25***a* of channel **25** is smaller than region **25***b* of channel **25**; beneficially, cap **200** can be configured so that it fits snugly within region **25***a* of channel **25**, i.e., the width of portion **200***a* of cap **200** is configured to fit within region **25***a* of channel **25**. When inserted within channel **25**, cap **200** is most preferably rigidly maintained therein. By "rigidly maintained" is meant that a user must take affirmative steps to remove cap **200** from device **100**, i.e., either by pulling cap **200** from channel **25** or by "screwing" cap **200** from channel **25**. The intent is that once cap **200** is within channel **25**, it will not be displaced therefrom due to, e.g., tipping of device **100**, etc. Cap **200** preferably comprises handle **210** for purposes of gripping thereof.

Neck portion **20** further comprises opening **27** into reservoir portion **10**. Opening **27** is configured to receive both the extraction liquid and a sample receiving platform. Advantageously, opening **27** can be covered with a removable, chemically inert material, for example a thin film of paraffin or plastic-backed foil (not shown). The intent of such material is to, e.g., prevent evaporation of the extraction liquid, and to add an extra measure of security vis-a-vis leakage of such liquid. Prior to insertion of a sample carrier through opening **27**, such material can be removed and, e.g., discarded or re-applied to opening **27** after insertion of the sample carrier into reservoir portion **10**.

At the opposite end of reservoir portion **10** relative to neck portion **20** is nozzle portion **30** which is most preferably angularly offset with respect to the longitudinal extent of walls **11** and **12**. By "angularly offset" is meant that wall portions **31** and **32** are not in the same longitudinal plane as walls **11** and **12** of reservoir **10** such that, for example, the width X between the walls **11** and **12** is greater than the width Y between the walls **31** and **32**. This configuration, therefore, has a somewhat "funnel" shape which descends to a leading end **35** of the nozzle portion **30**. The leading end **35** is configured such that an extraction liquid comprising sample can be released therefrom. The distance between portion **35***a* and **35***b* of leading end **35** is preferably spaced such that the liquid droplets exiting therefrom are capable of being adequately controlled. By "adequately controlled" is meant that, preferably, liquid does not rapidly exit the reservoir portion without inward pressure on walls **11** and **12**. Preferably, the distance between portions **35***a* and **35***b* is such that the surface tension of the liquid has a tendency to substantially remain within reservoir portion until released via applied pressure to walls **11** and **12**. Preferably, the distance between portions **35***a* and

35*b* is between about 0.02 and about 0.15 inches, more preferably between about 0.03 and about 0.06 inches, and most preferably about 0.047 inches.

Most preferably, leading end **35** further comprises an annulus **40** projecting in an upwardly direction into nozzle portion **30**. Annulus **40** assists in restricting and regulating the flow of liquid through leading end **35**. The opposing sides **40***a* and **40***b* of annulus **40** can be either substantially parallel or substantially non-parallel depending on the necessity for controlling the liquid flow. I.e., by directing the upper most portions **40***a* and **40***b* of annulus **40** towards each other (i.e., sides **40***a* and **40***b* are substantially non-parallel) the distance between **40***a* and **40***b* gradually decreases, thus decreasing the area for release of liquid.

In configurations of the device where the release of solid-type materials through leading end **35** is a consideration, screening means (not shown) can be positioned across leading end **35** (or, in an equivalent manner, across nozzle portion **30** and/or reservoir portion **10**) to prevent the release thereof. Screening means such as mesh are well known and will not be discussed herein in detail. Those in the art can readily select a screening means that is capable of being utilized in conjunction with biological and chemical materials, and which has sufficiently sized openings that allow for the release of liquid to the exclusion of particulate materials.

Positioned at the terminal end of leading end **35** is a sealing closure, generally depicted as **50** in FIGS. **1** and **2**. Sealing closure **50** is connected to leading end **35** by line of weakness **55** (FIG. **2**); when line of weakness **55** is broken, thus freeing sealing closure **50** from device **100**, a discharge opening (not shown) is presented from which materials within reservoir portion **10** can be released. Line of weakness **55** is preferably configured such that it is easily broken, for example, by gentle movement of sealing closure **50** along line of weakness **55**. However, in an equivalent manner, line of weakness **55** need not be broken by such movement, but can be cut using any conventional cutting tool. However, it is preferred that the former approach be utilized in order to avoid possible contamination and/or cross-contamination which could be occasioned by such a cutting tool.

Most preferably, sealing closure **50** is connected to base **60**; a purpose for base **60** is that it is capable of allowing device **100** to be positioned in an upright orientation, i.e., the device including base **60** is "free-standing". Such a configuration is beneficial because it can allow for introduction of a sample carrier to device **100** without the need to handle the device. The base **60** includes a rim or support surface **62** that is generally perpendicular to a central axis **64** of the vial **100**, to make the vial **100** "free-standing" as just described. Beneficially, support mechanism **70** can be positioned between base **60** and nozzle **30** for an added degree of rigidity and support; as is appreciated, when support mechanism **70** is utilized, the connection **71** between mechanism **70** of nozzle **30** must also be a line of weakness as described above such that mechanism **70** is removed from device **100** when sealing closure **50** and base **60** are removed via line of weakness **55**.

An extraction liquid (**80**) is most preferably included within reservoir portion **10** prior to insertion thereto of a sample carrier; however, in the case of, e.g., kits, extraction liquid can be included within another container and added to device **100** at an appropriate time. The extraction liquid is configured for use in the analysis of chemical or biochemical materials, such as those materials described above. Those skilled in the art are credited with readily selecting appropriate components for the extraction liquid in conjunction with the particular materials to be analyzed. For purposes of elucidation and not limitation, the following is directed to preferred extraction liquids utilized in conjunction with immunochromatographic assaying devices.

Generally, there are at least three criteria which must be considered when selecting an appropriate extraction liquid when used in conjunction with immunochromatographic assaying devices: (1) the extraction liquid must be compatible with a chemical or biochemical sample; (2) the extraction liquid must be compatible with the material used to manufacture vial **100**; and (3) the extraction liquid must be compatible with the diagnostic testing matrix.

The first criteria is satisfied by utilizing a solution having a suitable pH, i.e., a pH within the range of between about 2.0 and about 12.0, preferably between about 6.0 and 8.0, and most preferably about 7.4. Exemplary solutions include, but are not limited to, phosphate buffered solutions, imidazole-hydrochloric acid ("HCl"), tris hydroxymethyl amino methane ("Tris"), Tris-HCl, 2-{[tris(hydroxymethyl)methyl]amino} ethane sulfoni acid (TES), N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES), triethanolamine hydrochloride and the salts thereof, piperazine-N,N'-bis (2-ethane-sulfonic acid) (PIPES), N-2-acetamide-2 aminoethanesulfonic acid (ACES), 3-(N-morpholino propanesulfonic acid (MOPS) and combinations of the foregoing. Preferably the buffer is a phosphate buffered solution having a pH of about 7.4, most preferably, the phosphate buffered solution is phosphate buffered saline.

The second criteria can most efficiently be satisfied by utilization of a chemically inert material for the construction of vial **100**. Representative materials include, but are not limited to, polypropylene, polyvinylacetate polyethylene, polynitrile, polyethyleneterephthalate, polyethylene-vinyl acetate, polyethylene-vinyl alcohol, polyvinylchloride and combinations of the foregoing. Preferably, the material is polyethylene.

The third criteria is satisfied relative to the diagnostic testing matrix. With respect to immunochromatographic assaying devices, such matrices, by definition, have attached thereto immunological binding partners to analyte(s) in the sample. Thus, the extraction liquid must also be compatible therewith. As such, the same criteria (and buffers) set forth above for the first criteria are applicable to the third criteria. Additionally, it is typically necessary to include a non-specific binding inhibitor within the extraction liquid relative to the third criteria. Such a material functions to limit non-specific binding on the test matrix; exemplary materials include, but are not limited to, serum albumins such as, for example, human, bovine, goat, rabbit, sheep and horse serum albumins, ovalbumin, water soluble amino acid polymers, and combinations of the foregoing. Preferably, the non-specific binding inhibitor is bovine serum albumin, preferably within the range of about 0.5 to about 5.0% (weight/volume, "w/v"). When the diagnostic testing device is not an immunochromatographic device, such inhibitor is not required (although it can be utilized).

Accordingly, with respect to many immunochromatographic testing protocols, and in particular, protocols for the analysis of fecal occult testing via

hemoglobin analysis, a particularly preferred extraction liquid comprises phosphate buffered saline having a pH of about 7.4 and about 1.0% (weight/volume) of bovine serum albumin.

Beneficially, the extraction liquid can, and preferably does, further comprise a preservative, such as, sodium azide, thimerosal or sodium benzoate. The extraction liquid can further, and preferably does, comprise at least one surfactant. The purpose of the surfactant is two-fold: to assist in solubilization of the sample, and to assist in migration of the sample along the immunochemical test matrix. Exemplary surfactants include, but are not limited to, alkyl glucosides, betaines, bile acids, glucamides such as, for example, MEGA TM-8, -9 and -10 (available from Sigma Chemical Co.), and polyoxyethylenes, such as for example, the TRITON TM, GENAPOL TM (available from Hoechst Celanese), THESIT TM, BRIJ TM, TWEEN TM and PLURONIC TM surfactants (available from Sigma). Preferably, the surfactant is TRITON X 100.

The size of vial 100 is such that it is preferred that between about 0.2 ml and 2 ml of extraction liquid is included therein (or, in the case of kits, added thereto). Most preferably, about 0.4 ml of an extraction liquid comprising phosphate buffered saline (pH 7.4), 1% bovine serum albumin (w/v), 3.6% Triton X-100 (volume/volume), and 0.1% (w/v) sodium azide is utilized.

The extraction vial can further incorporate additional features consistent with the objectives of the vial. For example, antibodies specific for interfering analytes (i.e., analytes in the sample that are preferably not added to the diagnostic matrix) can be covalently attached to the inner walls of the vial. The intent of such antibodies is to "remove" such interfering analytes from the liquid which is released into the matrix. Methodologies for covalently attaching antibodies to materials such as polyethylene are well known and will not be discussed herein in detail. Additionally, the extraction liquid itself can include (or can have added thereto), soluble antibodies to the desired analyte such that upon introduction of the test sample-comprising analyte, the soluble-antibody:analyte conjugate can be released onto the matrix. The extraction liquid can also comprise (or have added thereto), insolubilized antibody specific for interfering analytes; insolubilizing materials, such as latex particles, etc., are well known and will not be set forth herein detail. In such a configuration, it is preferred that a mesh material be utilized such that the insolubilized materials cannot pass through (or clog) the leading end of the nozzle.

Other materials can also be utilized in conjunction with objectives of the vial. For example, with fecal occult blood ("FOB") screening for colo-rectal cancer, it is known that blood from the lower gastrointestinal ("GI") tract may be indicative of such cancer, while blood from the upper GI tract (which may be indicative of an ulcer but which is not indicative of such cancer) can lead to so-called false-positive tests. I.e., for FOB screening, typically only lower GI blood is of import. It is also known that as blood passes through the stomach, hydrochloric acid converts the relatively uncharged hemoglobin in the blood to hematin and related hemoglobin breakdown products ("HBPs"); these are highly charged. Because blood from the lower GI tract has not passed through the hydrochloric acid in the stomach, the hemoglobin therein is not charged. These facets can be beneficially exploited.

For example, the extraction vial and/or liquid can include (or have added thereto) an ion exchange material such that hematin and HBPs which may be present in the sample are selectively attracted thereto via a charge interaction. Hemoglobin from lower GI bleeding, which is not charged, is not attracted to such material. Accordingly, use of such materials allows for the selective release of extraction liquid comprising (if present) lower GI tract hemoglobin onto the matrix. This protocol provides a highly specific screening protocol for colo-rectal cancer.

Ion exchange materials are well known and will not be discussed herein in detail. Materials which can serve as ion exchange media typically possess, but are not limited to, media with functional groups such as: alkylcarboxylates, alkylsulfonates, arylsulfonates, primary alkylamines, secondary alkylamines and quaternary amines. Specific ion exchange materials are available from Analytichem International, Inc. (Harbor City, Calif.); particularly preferred ion-exchange materials are, e.g., PSA, SAX and PRS. PSA is an anion exchanger, where the functional group is ethylenediamine-N-propyl; SAX is an anion exchanger, where the functional group is trimethylaminopropyl (chloride form); and PRS is a cation exchanger, where the functional group is sulfonylpropyl (sodium form).

While the foregoing has been described in considerable detail, it is to be understood that the foregoing description and drawings of preferred embodiments are not to be construed as limiting the disclosure or the claims to follow. Modifications which are within the purview of the skilled in the art are included with the scope of the disclosure and the claims to follow.

What is claimed is:

1. A vial suitable for use with an extraction liquid, comprising:
   - a reservoir portion defining a chamber consisting of a pair of spaced, flat, generally parallel walls, the walls being compressible;
   - a neck portion integral with the reservoir portion at a first end of the reservoir portion, the neck portion defining an opening into the reservoir portion and a channel surrounding the opening;
   - a nozzle portion integral with the reservoir portion at a second end of the reservoir portion, the nozzle portion narrowing to a leading end and including a passage connecting with the chamber and the leading end;
   - a sealing closure connected to the leading end by a line of weakness so as to close the leading end;
   - a base integral with the sealing closure located opposite from the nozzle portion, the base including a support surface generally perpendicular to a central axis of the vial for stably supporting the vial in a generally vertical, upright position;
   - a support tab integral with and extending between the nozzle portion and the base; and
   - a cap including a portion adapted to be snugly received and removably rigidly maintained within the channel.

2. A vial as in claim 1 wherein the walls of the chamber include means for providing a gripping surface on the exterior of the walls.

3. A vial as in claim 1 wherein the vial further comprises an extraction liquid in the chamber of the reservoir portion adapted for use in the analysis of biological or chemical materials.

4. A vial as in claim 1, the nozzle portion further comprising an annulus projecting upward into the passage and located at the leading end of the nozzle portion.

* * * * *

US005413708A

United States Patent [19]

Huse et al.

[11] Patent Number: 5,413,708

[45] Date of Patent: May 9, 1995

[54] **PUSH COLUMN CHROMATOGRAPHY APPARATUS**

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[73] Assignee: **Stratagene**, La Jolla, Calif.

[21] Appl. No.: **232,713**

[22] Filed: **Apr. 25, 1994**

Related U.S. Application Data

[60] Division of Ser. No. 84,533, Jun. 28, 1993, Pat. No. 5,378,360, which is a division of Ser. No. 827,995, Jan. 30, 1992, Pat. No. 5,378,359, which is a continuation of Ser. No. 292,808, Jan. 3, 1989, abandoned.

[51] Int. Cl.[6] **B01D 15/08**

[52] U.S. Cl. **210/198.2**; 210/541; 422/104

[58] Field of Search 536/25.4; 210/635, 656, 210/198.2, 282, 416.1, 472, 541; 604/187, 190, 191; 436/161, 178; 422/70, 100, 101; 73/864.16, 864.17, 864.18, 864.81, 864.82, 864.83, 864.84, 864.85, 864.86, 864.87

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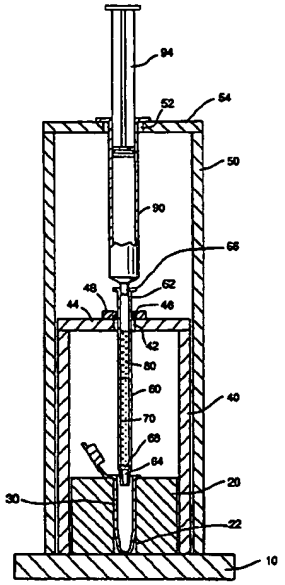
Primary Examiner—Ernest G. Therkorn

Attorney, Agent, or Firm—Limbach & Limbach

[57] **ABSTRACT**

An apparatus for chromatography of DNA, RNA, proteins and other molecules includes the use of a column adapted to hold a chromatography material and a sample to be filtered. A pneumatic pressure differential is applied across the column and the sample is urged through the chromatography material. A selected portion of the sample may then be collected.

5 Claims, 2 Drawing Sheets



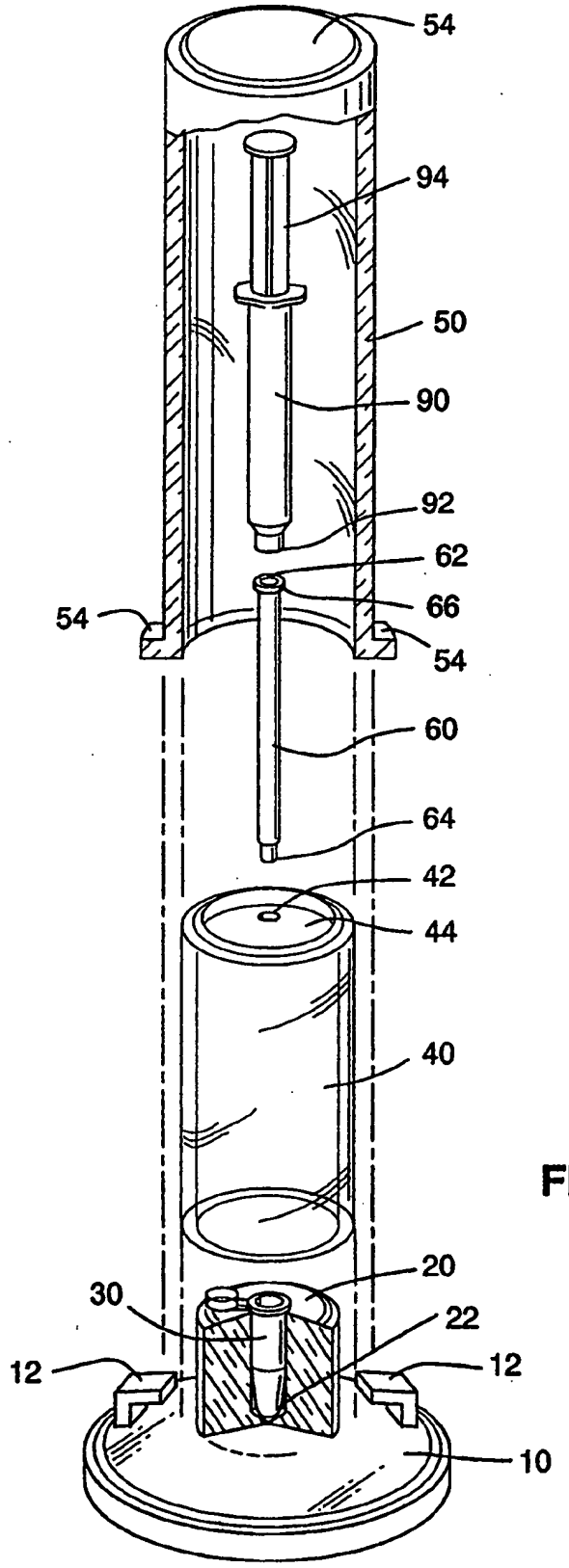


FIG. 1

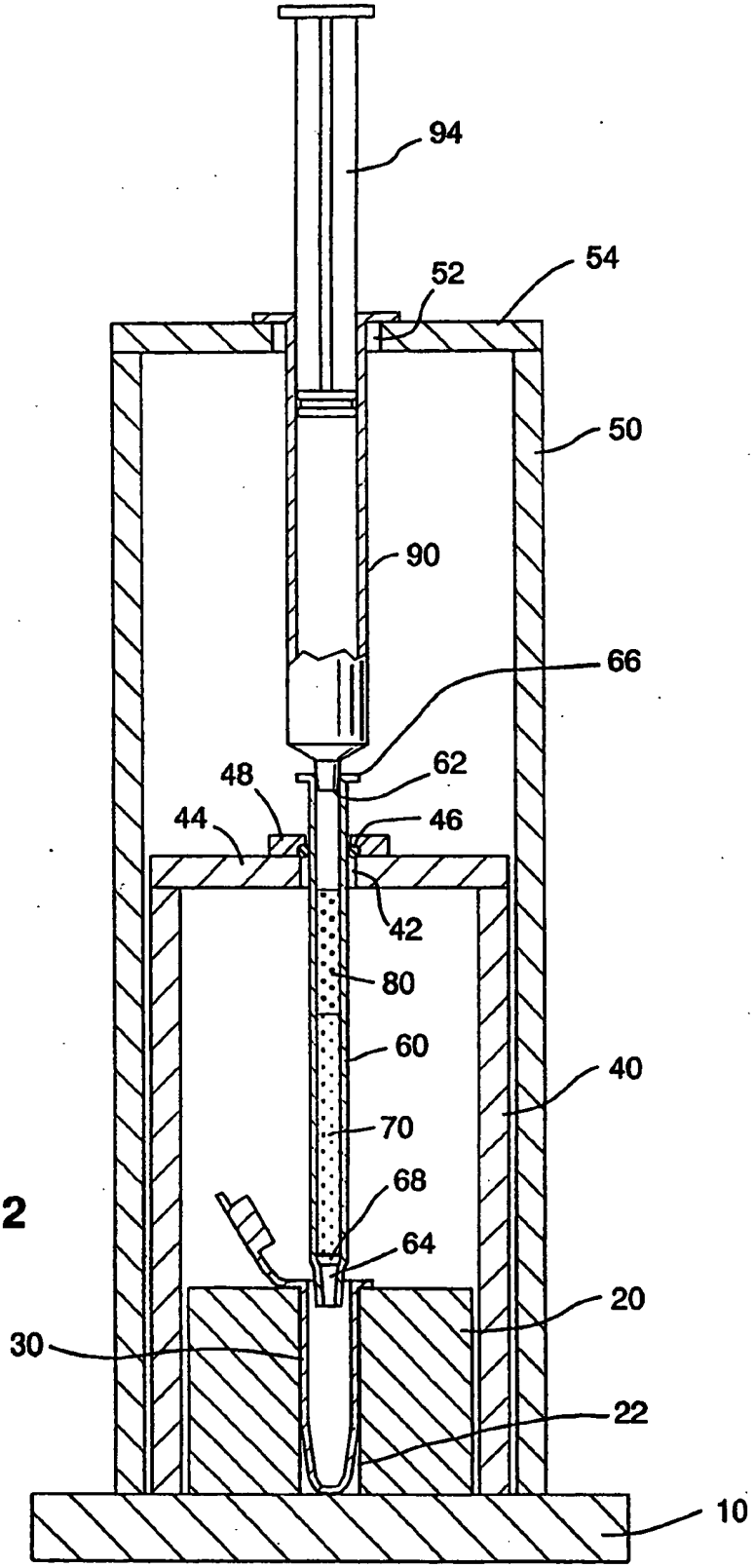


FIG. 2

PUSH COLUMN CHROMATOGRAPHY APPARATUS

This application is a divisional of application Ser. No. 08/084,533, filed Jun. 28, 1993, now U.S. Pat. No. 5,378,360, which is a divisional of application Ser. No. 07/827,995, filed Jan. 30, 1992, now U.S. Pat. No. 5,378,359, which is a continuation of application Ser. No. 07/292,808, filed Jan. 3, 1989, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an apparatus and methodology for the chromatography of materials, and in particular, chromatography based on molecular size, affinity and the like as used, for example, in the purification, separation or isolation of DNA and RNA fragments, proteins and other molecules.

2. Background Art

Removing unincorporated nucleotides from DNA and RNA fragments, isolating RNA fractions, purifying proteins and other macromolecules, are important procedures having a variety of applications. In DNA and RNA synthesis, unincorporated nucleotides must often be removed when constructing nick-translated probes, RNA probes and end-labeled oligonucleotides, as well as "filled-in" DNA fragments. It is important to separate the unincorporated free-nucleotides from the labeled probe as unincorporated label may bind to the solid support, resulting in unacceptably high levels of background noise. Isolation of RNA fractions may be employed in the separation of, for example, polyadenylated RNA from nonpolyadenylated RNAs. The use of chromatography methods to isolate and identify proteins and other macromolecules is another well known application.

Current chromatography methods, used particularly in connection with DNA and RNA synthesis, include ion-exchange chromatography several variations of eel chromatography and others. Each has its own disadvantages. For example, ion-exchange methods require a number of steps which may result in a significant investment of time and, in the case of radio-labeled nucleotide filtering, extensive handling of radioactive material. Conventional gel-chromatography "drip" columns are tedious, requiring time to both pour and run. Spin columns, a variation of the "drip" column, are somewhat faster, but risk radiation exposure and contamination in the case of radionucleotide chromatography, and may yield less reliable results.

An alternative chromatography approach which avoids the aforementioned difficulties would therefore be desirable.

SUMMARY OF THE INVENTION

The present invention is directed to an apparatus and method for purifying, isolating and separating materials using gel chromatography. To that end, a chromatography material and a sample may be loaded into a column and pneumatic pressure applied to urge the sample through the chromatography material, whereby portions of the sample may be collected by the chromatography material and other portions excluded. In one embodiment, a positive pneumatic pressure is provided and in a second embodiment a negative pressure is applied. Additionally, a novel support structure may be employed to support the column during chromatography. The sample may thus be quickly and reliably treated.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is an exploded perspective view of an apparatus constructed in accordance with the present invention comprising a column, pressure inducing means, a collection vial and associated support structure.

FIG. 2 is a cross-sectional view of the apparatus of FIG. 1 in a loaded position.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Referring to FIGS. 1 and 2, a chromatography apparatus constructed in accordance with the present invention comprises a generally disk shaped base 10 having a pair of retainers 12 and a generally cylindrical vial holding assembly 20 mounted thereon. Centrally located in the vial holding assembly 20 is a cylindrical chamber 22 for supporting a collection vial 30, into which the eluent from the column may be collected. The vial 30 may be a decapped Eppendorf tube or other suitable collection means. Removably mounted to the base 10, and slideably engaging the exterior wall of the vial holding assembly 20, is a generally cylindrical column support assembly 40. The column support assembly 40 includes a central aperture 42 formed in the generally planar upper surface 44 thereof. As shown in FIG. 2, the support assembly may have a resilient collar 46, such as an "O" ring or the like, positioned circumferentially adjacent the aperture 42, and a collar retainer 48 adapted to retain the collar 46 adjacent the aperture 42. Alternatively, as shown in FIG. 1, the collar 46 and the retainer 48 may be eliminated.

Optionally, a generally cylindrical pressure inducing means support assembly 50 may be removably mounted on the base 10. The support assembly may comprise a central aperture 52 formed in the generally planar upper surface 54 thereof, and is configured to slideably engage the exterior wall of the column support assembly 40. The aperture 52 is preferably axially aligned with the aperture 42 in the column support structure 40, which itself is preferably axially aligned with the chamber 22 in the vial holding assembly 20.

Alternatively, as shown in FIG. 1, the support assembly 50 may include an upper surface 54 having no aperture therein. The support assembly 50 may be further provided with a pair of locking tabs 54 adapted to engage the retainers 12 on the base 10 to lock the support assembly 50 in place during use. Other suitable locking mechanisms, such as threads, could also be employed. The assemblies 20, 40 and 50 may be formed of a radiation shielding material or, preferably, are constructed to fit securely inside a beta shield device. Molded plastic materials have been found suitable although other materials may also be employed.

Supported by the column holding assembly 40 above the vial 30 is a substantially tubular chromatography column 60. The column 60 may be about 1 ml in size, having a preferred internal diameter of about 5 mm and a preferred length of about 100 mm, and comprises openings 62 and 64, respectively, at each end thereof. An annular lip 66 may be provided circumferentially adjacent the upper opening 62, as shown in FIG. 1. The upper opening 62 is adapted to receive a chromatography material 70 and a sample 80 to be filtered. The lower opening 64 has an area of reduced cross-section adapted to prevent passage of the chromatography

material 70 while permitting passage of the sample 80. Additionally, a screen or filter 68, comprising, for example, glass wool, may be employed to retain the chromatography material 70 within the column 60. Preferably, for a tubular column, the internal diameter should not exceed about 10 mm for most chromotography applications, lest the surface tension of the sample be insufficient to prevent effervescence and consequent loss of pressure through the sample. Increasing the length of the column 60 should enhance the degree of separation. Preferably, the column should be no less than about 60 mm in length.

Pneumatic pressure inducing means 90, in this case a syringe, may be attached to the upper opening 62 of the column 60. As shown in FIG. 1, the syringe 90 may include a series of threads 92 which engage the annular lip 66 of the column 60 to retain the column 60 and the syringe 90 in mutual engagement. In a first embodiment of the invention, the syringe 90, having the plunger 94 withdrawn as shown in FIG. 2, may be attached to the column 60 and a positive pneumatic pressure differential applied between the openings 62 and 64 of the column 60 by depressing the plunger. In that case, the support assembly 50 may be placed over the support assembly 40. As the assembly 50 is lowered, its upper surface 54 (if no aperture 52 is provided) will contact the plunger 94 and automatically depress same until the bottom of the support assembly 50 meets the base 10. At that point the support assembly 50 may be twisted until the locking tabs 54 mate with the retainers 12. If an aperture 52 is provided in the support assembly 50, the plunger 94 will extend therethrough and may be manually depressed. In an alternative embodiment, not shown, the pressure inducing means 90 may be attached to the lower opening 64 of the column (the column may be removed from the support assembly 40 in that case) and the sample drawn through the column by a negative pressure differential between the openings 62 and 64.

The chromatography procedure may be commenced by removing the column holding assembly 40 from the base 10 and inserting the collection vial 30 into the aperture 22 in the vial holding assembly 20. The column holding assembly 40 is then returned to the base 10. The column 60 is inserted into the aperture 42 in the column holding assembly 40 and positioned so that the lower end of the column extends into the collection vial 30. An appropriate chromatography material may then be introduced into the column 60 using the syringe 90, or other suitable means.

If unincorporated nucleotides are to be removed from DNA or RNA fragments, gel chromatography material such as a polysaccharide or polyacrylamide, having a selected degree of internal porosity, may be employed. The sample containing DNA or RNA fragments (large molecules) and unincorporated nucleotides (smaller molecules) may be introduced into the top of the column 40 using a suitable pipetting device. Capillary action draws the sample into the upper portion of the chromatography material, i.e., between the "beads" comprising the material as shown in FIG. 2. Preferably, if a 1 ml column is employed, about 10–50 ul, preferably 50 ul, and no more than about 200 ul, of sample may be introduced. With the column thus prepared, the syringe 90 may be attached with the plunger fully extended to the column 20. The plunger may then be firmly depressed (with or without use of the support assembly 50) until the sample is pushed through the column into the collection vial 30. If the support assembly 50 is employed, the support assembly locking mechanism can be actuated to retain the plunger in a fully depressed condition. As the sample proceeds through the chromatography material, the smaller molucules, for example, unincorporated nucleotides, are partitioned into the pores in the chromatography material while the large molucules, for example, DNA or RNA fragments, are excluded. The eluent from the column should be substantially free of unincorporated nucleotides.

A similar procedure may be employed for affinity chromotagraphy applications such as hybridization of complimentary strands of nucleic acids. For example, to separate polyadenylated RNA from nonpolyadenylated RNA, oligo(dT)-cellulose may be employed as a chromatography material. Under appropriate buffer conditions, the desired polyadenylated RNA will bind with the oligo (dT)-cellulose chromatography material while the nonpolyadenylated RNAs will be eluted into the collection vial 30. The polyadenylated RNA can be recovered by a second buffer condition. Other affinity chromotography applications include the purification of specific nucleic acid sequences, for example, viral genomic sequences, by generating complementary oligonucleotides.

Thus, an apparatus and chromatography method employing a pneumatic pressure differential have been disclosed. While embodiments and applications of this invention have been shown and described, it would be apparent to those skilled in the art that many more modifications are possible without departing from the inventive concepts herein. For example, although purification of DNA and RNA fragments and separation of polyadenylated from nonpolyadenylated RNAs has been disclosed, many other chromotography applications would be possible. The invention, therefore, is not to be restricted except in the spirit of the appended claims.

What is claimed is:

1. An apparatus for supporting a chromatography column pressurized by a syringe and for supporting a collection vial at a discharge end of the column, the apparatus comprising:
   - a base;
   - a vial support coupled to the base and having a chamber therein adapted for receiving and supporting a collection vial;
   - a column support including a first hollow cylinder with an open lower end that slidably couples to the vial support on the base and an upper end with a hole therein adapted to support the column; and
   - a syringe support including a second hollow cylinder with an open lower end that slidably couples to the column support and an upper end adapted to engage the syringe.

2. The apparatus as recited in claim 1 wherein the column has a flange at an upper end thereof and said column support engages the column flange to longitudinally support the column, and wherein said syringe support has an upper surface inside the cylinder that engages the top of a plunger of the syringe so that the syringe support displaces the plunger downward when the syringe support is displaced downward.

3. The apparatus as recited in claim 2 further comprising means for releasably locking the syringe support to the base when the syringe support is displaced downward into contact with the base.

4. The apparatus as recited in claim 1 wherein said hole in the upper end of the column support laterally

supports the column, and wherein the syringe has a flange at an upper end thereof and said syringe support includes a hole in the upper end that engages the syringe flange to longitudinally support the syringe.

5. The apparatus as recited in claim 1 wherein said column support and said syringe support are composed of radiation shielding materials.

* * * * *

US005266193A

United States Patent [19]

Kimura et al.

[11] Patent Number: 5,266,193

[45] Date of Patent: Nov. 30, 1993

[54] **SYRINGE TYPE COLUMN FOR CHROMATOGRAPHY**

[75] Inventors: **Masaru Kimura**, Okayama; **Hiromi Kochi**, Fukuyama, both of Japan

[73] Assignee: **Manac Inc.**, Hiroshima, Japan

[21] Appl. No.: **932,972**

[22] Filed: **Aug. 20, 1992**

Related U.S. Application Data

[60] Division of Ser. No. 764,974, Sep. 23, 1991, Pat. No. 5,186,839, which is a continuation of Ser. No. 459,828, Jan. 26, 1990, abandoned.

[30] **Foreign Application Priority Data**

Jun. 3, 1988 [WO] World Int. Prop. O. PCT/JP88/00539

[51] **Int. Cl.**[5] **B01D 15/08**

[52] **U.S. Cl.** **210/198.2**; 210/656; 604/190

[58] **Field of Search** 210/635, 565, 198.2, 210/416.1, 472; 604/187, 190; 128/218; 436/161, 178; 422/70, 100, 101

[56] **References Cited**

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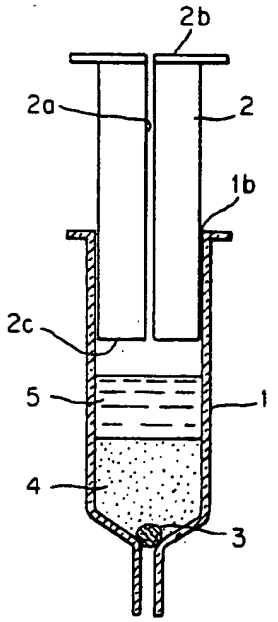
Primary Examiner—Ernest G. Therkorn

Attorney, Agent, or Firm—Frishauf, Holtz, Goodman & Woodward

[57] **ABSTRACT**

A syringe type column used for liquid chromatography, wherein a stationary phase (4) and a solvent for development (5) are placed in a cylindrical barrel (1), and an air vent (2*a*) extends through a plunger (2) which is inserted in the barrel. Development is effected, while the air vent (2*a*) is closed to prevent air leakage therethrough, by pushing down the plunger (2). When the plunger (2) is drawn out in order to supplement the solvent for development, air flows into the cylindrical barrel (1) through the air vent (2*a*) so that an inner space of the barrel (1) is not held under a negative pressure and the stationary phase (4) is not disturbed.

9 Claims, 2 Drawing Sheets



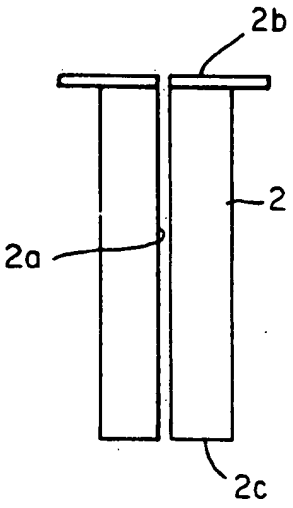


Fig. 2

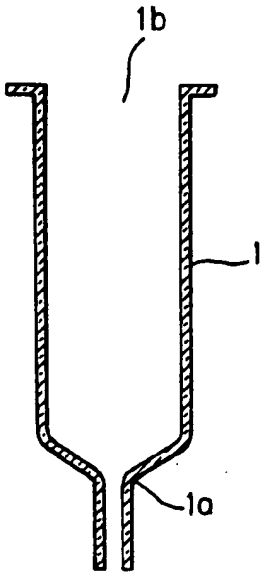


Fig. 1

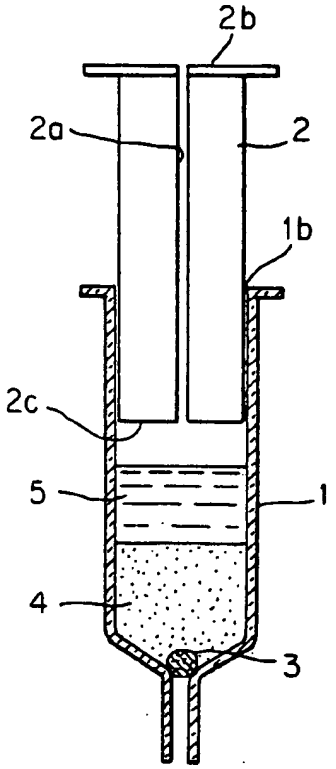


Fig. 3

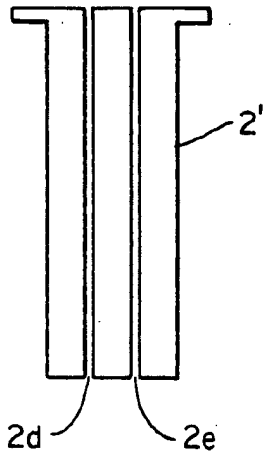


Fig. 4

SYRINGE TYPE COLUMN FOR CHROMATOGRAPHY

This is a division of application Ser. No. 07/764,974 filed Sept. 23, 1991, now U.S. Pat. No. 5,186,839 which is a continuation of Ser. No. 07/459,828 filed Jan. 26, 1990, now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to a syringe type column to be used for chromatography, and more particularly to a syringe type column having a novel structure, in which the uniformity in a stationary phase filled in a column may not be disturbed even after repeating operations of introducing a solvent for development thereto, so that the stationary phase filled in the column may not be disorganized or disturbed even after performing operations where high separating efficiency are required.

With recently required precision of chemical reactions, development of simple and convenient methods for purifying or separating a trace of a reaction product is becoming desideratum. As one of such methods, there has been contemplated a pressurized chromatography technique applied with a medium pressure in which a syringe is used as a column. In this method, a barrel of the syringe having a discharge port at the tip is first filled with a silica gel granule, an alumina granule or a cellulose fiber, to which a predetermined amount of a solution containing the reaction product to be separated is then introduced, and further a predetermined amount of a solvent for development. Subsequently, a plunger is inserted in the barrel to be pressed thereinto gradually to effect development and separation of a desired object, which is sampled from the discharge port of the barrel.

When high separating efficiency is tried to be obtained using this method, the following inconvenience occurs.

For the purification or separation of the reaction product, it is generally necessary to introduce the solvent for development repeatedly to the stationary phase while uniformly maintaining it in the filled state. However, in the above method, the plunger must be drawn out of the barrel every time the solvent for development is introduced into the barrel. When the plunger is drawn out of the barrel, an inner space of the barrel inevitably suffers a negative pressure, so that the air flows into the barrel through the discharge port at the tip thereof, and the filled state of the stationary phase is subject to turbulence due to the movement of the air flowing into the barrel to break down an equilibrium state formed therein.

It is an object of this invention to provide a syringe-type column having a structure in which the solvent for development can repeatedly be introduced into the column while the equilibrium state formed in the stationary phase is maintained and without causing the inconvenience as described above in said repeated introduction of the solvent for development.

SUMMARY OF THE INVENTION

The syringe type column of this invention comprises a cylindrical barrel having a solution discharge port at one end and an opening at the other end; and a plunger which is inserted from the open end of the cylindrical barrel and has at least one air vent formed to extend therethrough along the direction of an axis thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows, in longitudinal cross-section, a cylindrical barrel which is one member of the column of this invention;

FIG. 2 shows, in longitudinal cross-section, a plunger which is the other member of the column of this invention;

FIG. 3 is an illustration for explaining the operation of the column of this invention; and

FIG. 4 shows another embodiment of the plunger of this invention.

DETAILED DESCRIPTION

The column of this invention will now be described in more detail referring to the drawings. FIG. 1 and FIG. 2 show, in longitudinal cross-section, the cylindrical barrel and the plunger, respectively, which constitute a column when they are combined with each other.

The cylindrical barrel 1 has, on the whole, for example, a cylindrical shape, wherein the solution discharge port 1*a* is provided at a bottom end and wherein an upper end is defined as the open end 1*b*. The plunger 2 has at least one air vent 2*a* formed to pierce or extend therethrough along the longitudinal axis thereof from an upper surface 2*b* to a lower surface 2*c*.

The column of this invention is operated as follows: As shown in FIG. 3, the outlet of the solution discharge port 1*a* of the cylindrical barrel 1 is sealed with a liquid-permeable member 3 such as cotton. Next, a predetermined amount of a stationary phase 4 is filled into the barrel 1. As the stationary phase 4, a suitable one may be selected depending on the purpose from those used for ordinary column chromatography and HPLC. Subsequently, a predetermined amount of sample solution is introduced from the open end 1*b* and then a predetermined amount of a solvent for development 5 is further introduced.

The plunger 2 is then inserted in the open end 1*b* to be pressed into the barrel 1 with finger pressure of an operator being applied to the upper surface 2*b* thereof.

As the plunger 2 is pressed into the barrel 1, the solvent for development 5 permeates through the stationary phase 4, whereby a desired object can gradually be eluted. When the lower surface 2*c* of the plunger 2 substantially comes into abutment against an upper surface of the stationary phase 4 after consumption of the solvent for development 5, the finger applied to the upper surface 2*b* of the plunger 2 is released to draw out the plunger 2.

In the above drawing-out process, air flows into the barrel 1 through the air vent 2*a*, so that an inner space of the barrel 1 will never suffer negative pressure. Moreover, since the air flows in not through the solution discharge port 1*a* but through the air vent 2*a* of the plunger 2, the stationary phase 4 will never be subject to turbulence due to the movement of the in-flowing air.

FIG. 4 shows another embodiment of the plunger 2' in which two air vents 2*d* and 2*e* are formed to pierce or extend therethrough. In the plunger 2' of this embodiment, the solvent for development 5 can be introduced into the barrel 1 through the other air vent 2*e* without drawing out the plunger 2' therefrom.

The cylindrical barrels 1 and plungers 2, 2' may be made of glass or a resin such as polyethylene.

As is apparent from the above description, the column of this invention has very high practical value, since the solvent for development 5 can be introduced

thereinto repeatedly without breaking down the equilibrium state formed in the stationary phase 4 to thereby obtain high efficiency of separating the desired object.

We claim:

1. A syringe type column for use in chromatography sized and dimensioned for separation of a reaction product, comprising:

a generally cylindrical barrel having a solution discharge port at one end and an opening at another end thereof and a stationary phase suitable for ordinary column chromatography or HPLC uniformly filled in said barrel;

a liquid-permeable member closing said solution discharge port to the passage of the stationary phase;

a source of liquid solvent for development;

said barrel having respective volumes therein to be occupied with a stationary phase, a sample liquid solution containing a trace substance to be purified or separated therefrom, and a liquid solvent for development; and

a plunger which is repeatably insertable into and repeatably removable from said barrel, said plunger having at least one air vent formed therein, said at least one air vent extending through said plunger in a longitudinal direction of said plunger, said at least one air vent communicating the interior of said barrel with the outside of said barrel;

said at least one air vent being manually closable by an operator during insertion of said plunger into said barrel to cause said solvent for development to permeate through said stationary phase, and said at least one air vent being manually operable by the operator during drawing out of said plunger from said barrel for allowing air to flow into the interior of said barrel through said at least one air vent when said plunger is drawn out from said barrel and for thereby preventing said stationary phase from being subjected to turbulence due to the movement of in-flowing air through said solution discharge port during said drawing out of said plunger from said barrel.

2. The syringe type column of claim 1, wherein said plunger has a longitudinal axis, and said at least one air vent extends along said longitudinal axis.

3. The syringe type column of claim 1, wherein said plunger has a longitudinal axis, and said at least one air vent extends substantially parallel to said longitudinal axis.

4. The syringe type column of claim 1, wherein said plunger has an outer surface portion in sliding sealing contact with inner walls of said cylindrical barrel.

5. The syringe type column of claim 1, wherein said plunger has at least two spaced apart air vents formed therein and which extend therethrough in a longitudinal direction thereof.

6. The syringe type column of claim 5, wherein said plunger has a longitudinal axis, and said at least two air vents extend substantially parallel to said longitudinal axis.

7. The syringe type column of claim 5, wherein said plunger has an outer surface portion in sliding sealing contact with inner walls of said cylindrical barrel.

8. The syringe type column of claim 5, wherein said at least two air vents extend completely through said plunger from one end surface to an opposite end surface thereof.

9. The syringe type column of claim 1, wherein said at least one air vent extends completely through said plunger from one end surface to an opposite end surface thereof.

* * * * *

United States Patent [19]

Sisti et al.

[11] Patent Number: 4,526,686

[45] Date of Patent: * Jul. 2, 1985

[54] APPARATUS FOR CHROMATOGRAPHIC SAMPLE INJECTION

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[73] Assignee: Carlo Erba Strumentazione S.p.A., Rodano, Italy

[*] Notice: The portion of the term of this patent subsequent to Sep. 20, 2000 has been disclaimed.

[21] Appl. No.: 533,899

[22] Filed: Sep. 20, 1983

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 304,780, Sep. 23, 1981, Pat. No. 4,405,344.

[30] Foreign Application Priority Data

Sep. 30, 1980 [IT] Italy 25018 A/80
May 4, 1981 [IT] Italy 21504 A/81

[51] Int. Cl.³ B01D 15/08

[52] U.S. Cl. 210/198.2; 210/198.3; 55/197; 55/386

[58] Field of Search 210/658, 198.3; 55/67, 55/197, 386

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Primary Examiner—John Adee

Attorney, Agent, or Firm—Millen & White

[57] ABSTRACT

An apparatus for controllably and reproducibly introducing, small amounts of a liquid sample into chromatographic systems, especially high resolution gas chromatographic systems with cold injection, thin-layer chromatographic systems and high resolution liquid chromatographic systems, in order to obtain sampling extremely reduced in volume and presenting maximum reliability and reproducibility, uses a sample container having a pipette-like or nozzle-like outlet neck of very small diameter. The liquid placed in the container is submitted to at least one pressure pulse which is controlled in duration and/or amplitude in order to determine emission of a corresponding and controlled quantity of liquid from the outlet neck.

9 Claims, 9 Drawing Figures

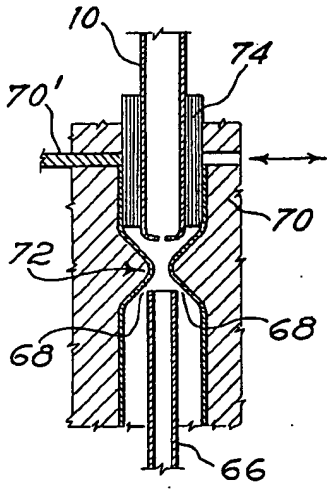


Fig. 1

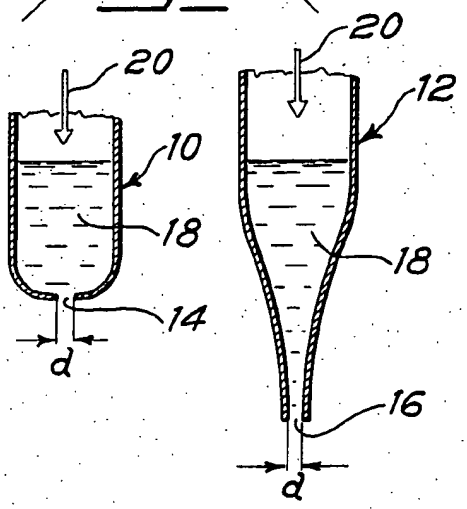


Fig. 2

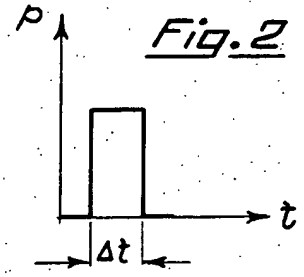


Fig. 3

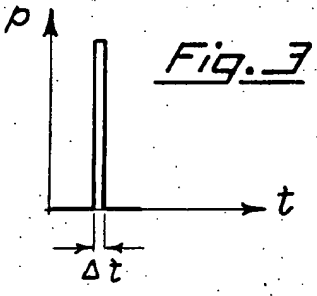


Fig. 4

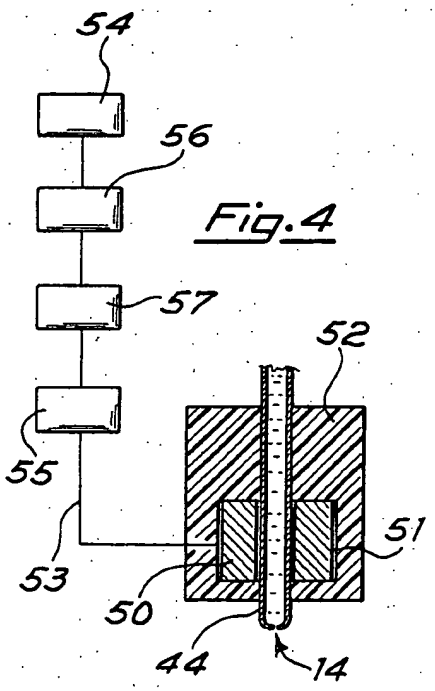


Fig. 5

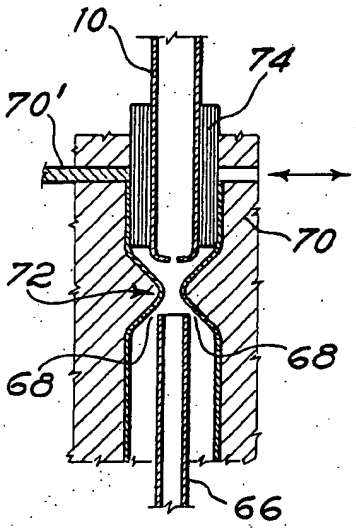


Fig. 6

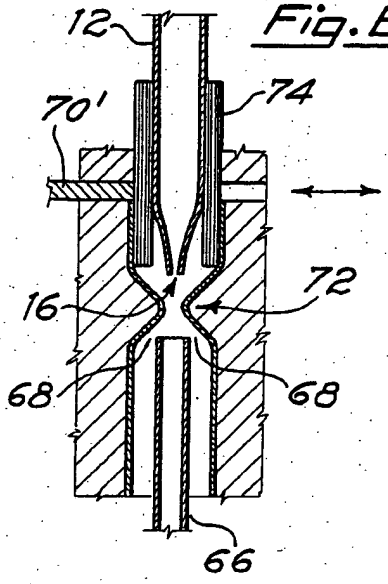


Fig. 7

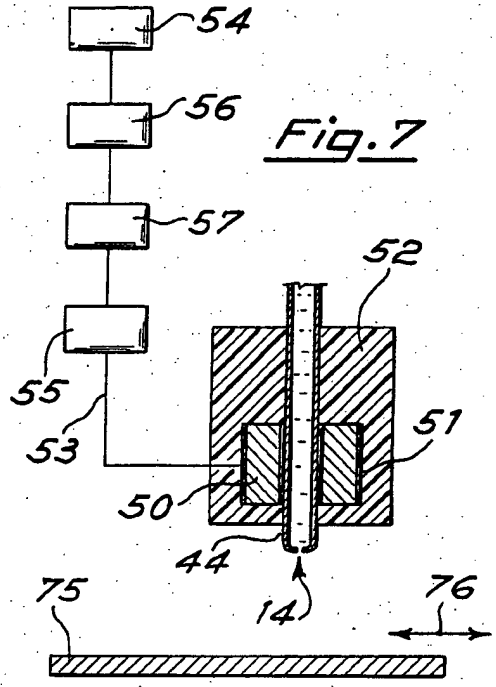


Fig. 9

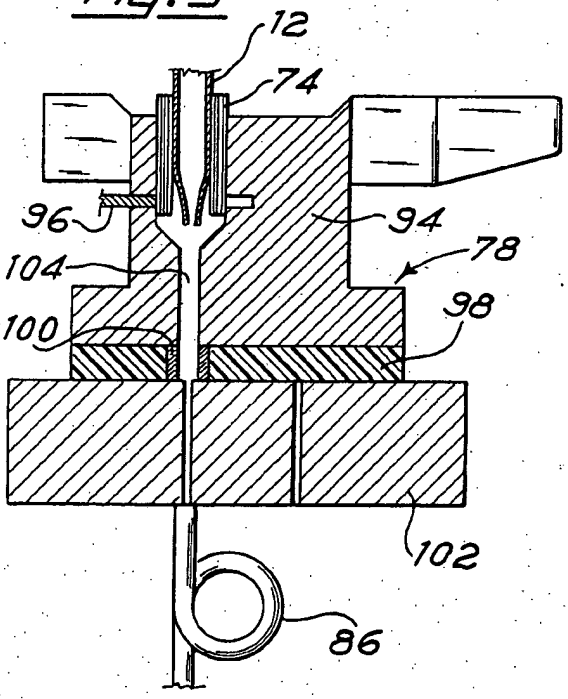
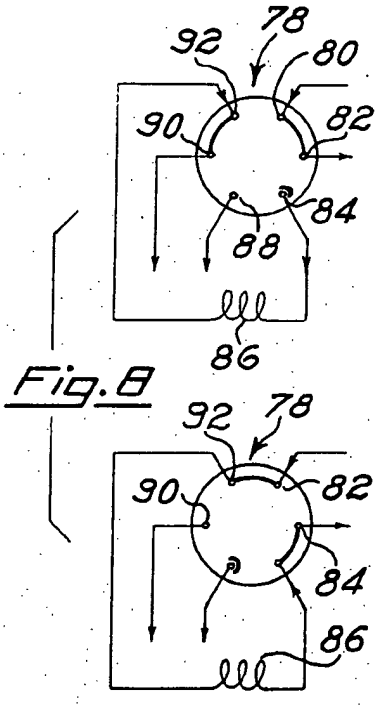


Fig. 8



APPARATUS FOR CHROMATOGRAPHIC SAMPLE INJECTION

This application is a continuation-in-part of U.S. Ser. No. 304,780, filed Sept. 23, 1981, now U.S. Pat. No. 4,405,344 issued Sept. 20, 1983, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an apparatus to perform sampling in chromatographic systems with very small amounts of liquid sample, said apparatus being particularly applicable to high resolution gas chromatographic systems with cold injection, using capillary or micropacked columns, to high resolution liquid chromatographic systems, or to thin-layer chromatographic systems. Use of this apparatus makes it possible to perform controllable and reproducible sampling on very small amounts of sample, with values unattainable through the techniques usually employed for liquid sampling in chromatographic systems and particularly using micro-syringes or pipettes.

2. Description of the Prior Art

For all chromatographic systems problems arise for injecting in a volumetrically controlled and reproducible way very small quantities of sample into a chromatographic system. In gas chromatographic systems with cold injection, when a cold on-column or a cold splitless injection (according for example to the method described in U.S. Pat. No. 4,383,839) are used, it has been foreseen the necessity to inject small sample amounts in order to reduce or eliminate the problems created by a high content of diluting solvent (flooding effect, partial solvent trapping effect etc). But also when cold split injection is considered a small sample is advantageous due to the fact that it requires a lower splitting ratio and then errors or discriminations generally arising at high splitting ratios are avoided (see for example K. Grob Jr. and H. P. Neukom—Journal of H R C & C C Vol. 2 September 1979; 563-569). The modern tendency to use small bore capillary columns also requires the injection of small sample sizes unachievable with the dosing systems used today.

In thin-layer chromatography the use of very small sample volumes is especially required (A. Zlakis and R. E. Kaiser: HPTLC Elsevier Scientific Publishing Company—Institute of Chromatography Bad Dürkheim 1977; 85-94).

Both these injection systems use syringes, microsyringes or pipettes as will be described later on.

The high resolution liquid chromatography systems usually use injectors with sampling valves having a loop wherein the sample is loaded for instance by means of a syringe. The loop is then connected with the column and with a source of eluting liquid solvent under pressure, to force the sample through the column.

In this case too, small volumes of sample are preferred and often imposed by the characteristic of the column. The modern trend is the use of microbore and capillary packed columns. Of course in all described injection systems it is necessary to obtain not only small sample quantities, but small sample quantities exactly measured and injected in a perfectly reproducible manner.

The microsyringes used in chromatography are generally of the type with calibrated body (capable of sampling amounts ranging from 0.2 to 10 microliters) or of the type with calibrated needle, where the piston penetrates into the needle. The latter microsyringes are capable of handling smaller quantities of samples, in a reliable and reproducible way, but only within certain limits, in particular with lower limits of about 100-200 nanoliters. Below this limit, the high surface tension of the liquid and the relatively reduced speed of the piston movement do not allow the drop, which has formed at the needle end, to fall from it, considering the reduced diameter of the outlet nozzle of the needle. Precision is moreover negatively affected by poor sealing between piston and calibrated needle. Another known system is the sampling system commonly used in the laboratory and named "pipette system", in which a calibrated tubing is filled with a liquid to be transferred by filling the pipette due to capillary forces or by sucking it into the tubing. The liquid amount placed in the tubing is retained in the tubing by the capillary forces or by closing one end when liquid aspiration has been carried out. Then, an injection of the liquid is made by opening said end or pushing the liquid by the carrier gas. This sampling method or transfer method of determined amounts of liquid is well known and has been used in gas chromatography too, but however only for quantities usually measurable in a rather rough way. The literature reports a lower limit of 25-50 nanoliters (see R. Kaiser—Gas Phase Chromatography—Vol. I pp. 90-95—Butterworths 1963—London) but thise limits are difficult to reach and anyhow require small tubes filled exclusively due to capillary forces. This implies that the volume of liquid injection is difficult to control and reproduce.

Therefore it can be considered that, of course according to the nature of the liquid substance to be sampled, a lower limit exists, generally between 50 and 200 nanoliters, below which it is not possible to go in reliable and reproducible manner using microsyringes or micropipettes.

The above mentioned quantitive limitations, however, are such that the operator is often forced to perform accessory operations imposed by the relatively high quantities of sample that has to be introduced into the chromatographic system. In particular, sometimes the sample must be diluted in a dilution ratio which is oftern very high (of the order of 1:10000 or more), with an operation which may involve difficulties in the exact analytical determination of the sample and in that it can introduce discriminations or variations in the sample original conditions.

In other cases, a splitting operation is necessary, that means the elimination of a high percentage of the quantity fed to the injector, before its introduction into the column, which operation may involve even higher risks of discriminations especially as above said, with hight split ratios. Between the known injection systems, Kaiser, *Gas phase Chromatography,* Vol. 11, pags. 59-62, discloses methods and apparatus for sample injection into gas chromatographic systems. Two particular types of injection devices are shown in FIGS. 26 and 27 of the reference. Both the injection devices described by Kaiser are versions of a micropipette type device. They are designed to be filled by capillarity with an uncontrollable quantity of liquid, which is then injected in its entirety into the evaporation area of sampling system. Sample sizes are in tenth of microliter range, roughly two orders of magnitude larger than the size of

sample which can be obtained according to the present invention.

The device of FIG. 27 of Kaiser is quite similar to that of FIG. 26. Again, the sample size is determined in the filling operation. This device is also capable of handling solids which can be melted to give a homogeneous liquid.

Taft et al., U.S. Pat. No. 3,366,149, discloses a system for injecting samples which is particularly useful for injecting larger samples for preparative gas chromatography. In this system, a constant pressure is maintained on the surface of a liquid in a sample container communicating with a heater through a valve. The valve is controlled by a solenoid pulser, which operates to open the valve for a controllable time interval.

This reference is relied upon for its showing of pulse flow. However, the pulse operates on the valve, and the reference does not show a pressure pulse operating on a liquid whose flow is not constrained by a valve. Rather than operating to inject samples in the picoliter or nanoliter range, the system of Taft operates to inject samples of the order of milliliters. The system is not designed to deal with very small sample sizes, and operates on an entirely different principle.

It will be seen from the above comparison that the prior art sample injection devices operate by controlling sample size in the filling step and injecting the entire contents of the filled injection system using pressure in a different way from the way in which it is employed in the present invention. Even a combination of the cited references would only lead the skilled art worker to a pipette of the type shown in FIG. 26 or 27 of Kaiser which, however, is actuated by a pulsed valve. This type of device is typical of a titration burette which commonly delivers minimum sample quantities of 0.1–0.2 milliliters, and is unable to deliver samples in the picoliter or nanoliter volume range.

OBJECTS OF THE INVENTION

Accordingly, an object of the invention is to provide an apparatus making it possible to perform sampling in chromatographic systems with small amounts of liquid sample; for instance from 10 picoliters to 50 nanoliters, in a reproducible way under the same conditions and sample type.

Another object of the present invention is to provide an apparatus, wherein the volumetric dosage of the substance injected into the chromatographic system is performed with the maximum of reliability, precision and reproducibility, during the injection stage itself.

SUMMARY OF THE INVENTION

According to the invention an apparatus is provided for volumetrically controlled and reproducible injection of small quantities of liquid sample into a chromatographic system comprising, in combination with an injection device for a chromatographic system:

a sample container having a volume grater than a desired liquid sample volume and having a pipette—or nozzle-shaped neck at its outlet, terminating in an opening with a diameter of 1–100 μm;

positioning means for operatively positioning said sample container in a sample injection device of a chromatographic system, for injection of a sample through said outlet opening; and

pulse means communicating with said container for applying to a liquid inside the container at least one pressure pulse of controlled amplitude and duration, and ending in an abrupt pressure drop; wherein said pulse means comprises at least one transducer capable of producing at least one pressure pulse of high amplitude and short duration in the sample container; a pulse source for exciting said transducer; and means for adjusting at least one of the amplitude or the number of the pulses.

Therefore, by such apparatus, it is now possible to carry out sampling of extremely reduced quantities of liquid, using a container which can be of the substantially traditional type, except for the outlet neck, to which a device can be applied which allows to create said pressure pulse, in such a way that the sample drawing and eventual washing of the container can be performed with extremely simple and traditional systems and means, through being possible to perform said samplings with extremely reduced quantities.

According to the invention said pulse means may comprise the application, to the container body and/or to means mechanically connected with the same, of a mechanical pulse, obtained with a piezoelectric system, a magnetostrictive system or other similar system, which determines an extremely high and extremely quick pressure increase in the liquid and allows the detention of short duration rectangular pulses.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an enlarged view, in axial section, showing possible shapes of the outlets of containers for samples to be used according to the invention.

FIGS. 2 and 3 are examples of possible pressure pulses used in the method and equipment according to the invention to perform desired sampling.

FIG. 4 diagrammatically shows an embodiment of apparatus for sampling by means of pressure pulses obtained with a piezioelectric system.

FIGS. 5 and 6 are diagrammatic views of possible positions of the sample container in use with a cold split-splitless injection.

FIG. 7 is a diagrammatic view showing the use of the apparatus in thin layer chromatography.

FIGS. 8 and 9 are diagrammatical views showing the use of the apparatus in liquid chromatography with a known sample loading device.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates, as already said, to an apparatus for sampling, in chromatographic systems, of liquid samples, said apparatus being particularly applicable to laboratory gas chromatography with cold split or splitless injection, in high resolution liquid chromatographic systems or in thin-layer chromatographic systems. This apparatus is capable of allowing injection of a very small liquid sample sizes compatible with the requirements of high resolution chromatographyc sistem, without need of dilution, as it is necessary in traditional method and apparatus to obtain a precise dosage of the injected sample. Although larger amounts can also be injected, the apparatus which will be illustrated is particularly suitable for the injection in a realiable and reproducible way, of liquid samples in quantities ranging between 10 picoliters and 50 nanoliters, i.e. amounts so small that they could not be handled with the previously employed systems.

The apparatus of the invention is based on the use of a container for the liquid sample which has a volume greater than that of the sample to be injected, said con-

tainer being made of any suitable material, for instance glass, metal, fused silica or any other material, and having a pipette shape, a syringe needle shape or any other suitable shape.

The essential condition is that said container, as indicated by **10** or **12** in FIG. **1**, presents a neck defining an outlet having a maximum size (a diameter d in this specific case) ranging from 1 to 100 μm, preferably between 1 and 30 μm. Said neck can be nozzle-shaped, as indicated by **14** in FIG. **1**, and therefore has a preferred neck diameter d from 10 to 30 μm, or micropipette-shaped, as indicated by **16** in the same FIG. **1**, with a preferred diameter d from 1 to 20 μm. The container, **10** or **12** as illustrated in FIG. **1**, can be filled by the usual methods, for instance by means of a syringe, by gravity, or in any way whatsoever, with a quantity of liquid **18** which is greater than the volume that is required to flow from the neck **14** or **16** to be analyzed in the chromatographic system. Once the container **10** or **12** has been fed with the sample **18**, it is necessary to check, especially in the case of the container **12** with a pipette-like end, that the liquid goes as far as to reach the neck **14** or **16**, forming a meniscus therein. Under these conditions, taking into account the reduced size of the neck, the surface tension of the liquid prevents the latter from flowing out of the neck, forming drops, this obviously provided that the neck **14** or **16** does not touch foreign bodies, which may help the liquid to flow outside the container.

Once the feeding of the container **10** or **12** as previously indicated has been carried out, pressure on the liquid **18** is exerted directly or indirectly as schematically shown by the arrows **20** in FIG. **1** to eject a mesured amount of liquid **18** through the neck **14** or **16**. Said pressure is controlled in amplitude and in time as indicated in FIGS. **2** and **3**, keeping into consideration that the liquid amount ejected depends both on the amplitude, namely the value of the pulse pressure, on the duration of the pulse itself and on the number of pulses.

As it can be seen from FIGS. **2** and **3**, the pulse has a step configuration and it is very important that the downwards section of same, namely pressure return to zero, be placed vertically as much as possible in order to avoid, during this stage, in correspondence to the neck **14** or **16**, the formation of a drop which will remain in position, thus completely altering any measuring of the injected sample. In the case of FIG. **2**, the pressure pulse is a long duration pulse and reaches a value which must be in any case higher than that necessary to overcome the surface tension of the liquid in correspondence with the neck **14** or **16**. In relation to the pressure value, the duration Δt of the pulse is chosen, variable from about a millisecond to about a second, in order to obtain the desired quantities of ejected liquid.

Alternatively, it can obsiously be possible to emit several pulses having lower duration. However, for an exact reproducibility of sampling, it is very important to reduce to the minimum the dead space, that is the gas volume on which each pressure pulse acts.

The situation illustrated in FIG. **3** is the one that occurs in case of a pressure pulse exerted by means of magnetostriction, or piezoelectricity. In this case, the time Δt is extremely reduced and corresponds to the resonance frequency of the laminations forming the magnetostriction device or to the frequency of the piezoelectric material, ranging from 10 to 100 KHZ, while a certain regulation of the quantity emitted at each pulse can be performed by varying the amplitude of the electric pulse given to the device and consequently the pulse pressure value given to the liquid, said pressure value being in any case of one or several orders higher than that necessary in the pneumatic case.

The sampling apparatus illustrated in FIG. **4** operates with a piezoelectric system which, in that part including the element for formation and emission of the jet of drops, is substantially configured like an inkdrop printing device, of the "jet on demand" type, known in itself. It essentially comprises a container **44**, for example but not necessarily with a cylindric shape, made of glass or fused silica, ready to be filled in its lower section with the sample to be injected into the column, for instance introduced through the upper section thereof.

The container **44** ends in its lower section with a calibrated nozzle **14**, as proviously indicated, through which the liquid jet of sample is emitted. Under atmospheric pressure conditions, the liquid does not flow out of the nozzle **14** because of its surface tension. The container **44** is connected to a transducer **50** of a piezoelectric type, capable of provoking, when excited, a sudden volume variation inside the container **44** and therefore a sudden pressure variation in the liquid present in it, such as to determine the flowing out of a calibrated jet of one or more drops through the nozzle **14**. The transducer **50**, which is placed very near to the nozzle **14** and housed for example in a small block **52** made of plastic material, is excited by means of a source of electrical pulses **54**, which have the characteristics previously described, feeding of the transducer **50** on its electrodes **51**, through a circuit **53** having a switch **55** by means of which the operator can close the circuit and then control the emission of the jet of drops. To the source **54** a known means **56** is connected to vary the amplitude of pulse or pulses communicated to the transducer **50**; another component **57** is also connected, capable of controlling the duration of the pulse or series of pulses.

For operation, the container **44** must be filled with the liquid sample, possibly by means of a microsyringe, at least in its section close to the nozzle **14**, and the transducer **50** is submitted to a pulse or series of pulses having a predetermined amplitude, during which, in correspondence with the nozzle **14**, a jet of one or more drops of sample liquid is emitted, in a small pre-set table quantity. Said jet presents high directionality and an extremely limited increase in diameter, so that it can be directed into a column with one the systems which will be considered, for example through the injecton port of a direct injector, equipped with a "slice" type or rotative valve.

The amplitude of pulses may be regulated by the element **56**, in order to obtain a corresponding regulation of the pressure increase provoked by said pulses and consequently of a first parameter affecting the operative conditions, in particular of the correct formation of the jet of drops, considering the nozzle diameter and the nature of the treated liquid. When the other conditions are kept unchanged, the ejected quantity of a given sample, thanks to the formation of a pulse having a preset amplitude, is exactly definite and equal to a drop, and therefore the ejected quantity obtained by a series of pulses will depend only on the duration of the latter and therefore on the number of pulses forming such series, because the time period of each pulse is determined by the transducer characteristics.

FIGS. 5 and 6 show the use of the injection apparatus in cold injection split or splitless gas-chromatography, for instance to carry out the method as depicted in the U.S. Pat. No. 4,383,839.

In this case, the injector body 70 shows a neck 72, which advantageously has an axial length as small as possible and on the two sides of which there are positioned a pre-column or vaporization chamber 66 and the container 10 or 12, the latter being preferably housed in the protecting collar 74, which provides for seating of said container 10 or 12 on the inner wall of the injector.

In this case, it is advisable, as already said, (i) that the distance between the outlet neck 14 or 16 of container 10 or 12 and the pre-column 66, as measured in an axial direction, be as small as possible, for instance 10 mm maximum, (ii) that the injection pressure of the jet into the neck 14 or 16 be sufficient for maintaining the latter in such a condition as to give rise to a very small opening angle of the jet, (iii) that the axial alignment of the neck 14 and pre-column 66 be perfect, and (iv) that a valve, f.i. a slide valve 70', be placed to close the injector duct when the injection device is not in position. It is particularly important in this case that, during injection, the carrier feeding, always according to arrows 68, be discontinued to avoid having the carrier gas drag the outside part of the injected sample, especially if the latter contains easily vaporizable substances. FIG. 7 diagrammatically shows the use of an injection apparatus as above described, in thin layer chromatography. The apparatus is the same as shown in FIG. 4 and the same components thereof are shown with the same reference numerals. The injector feeds a small volume of sample on a well known plate or dish 74 which is fixed when a radial elution is foreseen. As an alternative, the dish 74 and the injector body 52 may have a relative rectilinear movement, as shown by arrow 76 when a longitudinal elution in foreseen.

FIGS. 8 and 9 show an injection device as used in high pressure liquid chromatography. As well known, the injection device uses a valve with two bodies which can be axially rotated in order to put the same in at least two different positions, namely a load position and an injection position, as diagrammatically shown in the upper part and respectively in the lower part of FIG. 8. In the load position of valve 78, a pump connected at 80 feeds liquid under pressure to the column, which is connected at 82. A port for injecting the sample is shown at 84 and is connected with a loop 86 having its other end connected to the valve at 92. Ports 88 and 90 are connected to vents. In the load position the liquid from 80 enters the column in 82 and washes the same. A sample introduction is carried out at port 84. Then the valve is rotated in its injecting position and the liquid from 80 enters the valve, washes the loop 86 and injects the sample in the column. In this case too it is important to have very small sample volumes and to this end the sample introduction at port 84 can be carried out as depicted in FIG. 9 by means of a device 12-74 of the type of that shown in FIG. 6. The valve 94 has a stator 102 and a rotor 94 with a rotor seal 98 therebetween. A duct 104 with an interposed seal 100 crosses the three elements 94, 98 and 102 when the valve is in its load position. A slice valve 96 closes an enlarged outer part of duct 104, wherein the device 12-74 can be removably housed to inject a calibrated sample small volume within the duct 104 until reaching the loop 86. Of course other types of devices, for instance similar to that of FIG. 4 or that of FIG. 5 can be used.

Finally, it should also be noticed that the embodiments of the present invention as above illustrated and described can be submitted to several changes and variations without departing from the spirit and scop of the invention itself, these variations comprising the use of an automatic sampler, wherein the container is automatically filled and wherein sample emission occurs through several jets of sample.

We claim:

1. An apparatus for volumetrically controlled and reproducible injection of small quantities of liquid sample into a chromatographic system, comprising, in combination with an injection device for a chromatographic system;

a sample container having a volume greater than a desired liquid sample volume and having a pipette- or nozzle-shaped neck at its outlet, terminating in an opening with a diameter of 1-100 μm;

positioning means for operatively positioning said sample container in a sample injection device of a chromatographic system, for injection of a sample through said outlet opening; and

pulse means communicating with said container for applying to a liquid inside the container at least one pressure pulse of controlled amplitude and duration, and ending in an abrupt pressure drop; wherein said pulse means comprises at least one transducer capable of producing at least one pressure pulse of high amplitude and short duration in the sample container; a pulse source for exciting said transducer; and means for adjusting at least one of the amplitude or the number of the pulses.

2. An apparatus according to claim 1, wherein said injection device is a gas chromatography injection device comprising a cold sample split-splitless sampling system in which the vaporizing chamber is maintained cold during the liquid injection and thereupon heated to vaporize the sample.

3. An apparatus according to claim 1, wherein said injection device is used to deposit the sample on thin layer chromatography plate.

4. An apparatus according to claim 1 wherein said injection device is a high resolution liquid chromatography injection device of the valve type containing a loop with a larger volume in which the small sample size is injected.

5. An apparatus according to claim 1, wherein said transducer is a piezoelectric transducer, and said pulse source is a source of electric pulses of high amplitude and steep dropoff.

6. An apparatus according to claim 5, wherein said sample container is made of glass or fused silica and is surrounded, near the outlet opening thereof, by a piezoelectric transducer embedded in a small block of a plastic material.

7. An apparatus according to claim 1, wherein the sample container has at least a part thereof made of nickel in the portion containing the liquid sample; wherein the transducer comprises a magnetostrictive device capable of acting on said container part when in its operative position within a seat provided in said magnetostrictive device, and actuating means for applying to said magnetostrictive device at least one current pulse having an adjustable amplitude, thereby producing a corresponding pressure pulse acting on the liquid contained in the nickel part of said container.

8. An apparatus according to claim 7, wherein said pulse has a duration which depends on the frequency of mechanical resonance of the magnetostrictive device and is adjustable in amplitude.

9. An apparatus according to claim 1, wherein the diameter of the sample container outlet opening is 1-30 μm.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,526,686

DATED : July 2, 1985

INVENTOR(S) : GIORGIO SISTI ET AL

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page, second inventor: read "Sorin Trestiano"

should read -- Sorin Trestianu --.

Signed and Sealed this

Fifteenth Day of *October 1985*

[SEAL]

Attest:

Attesting Officer

DONALD J. QUIGG

Commissioner of Patents and Trademarks—Designate

United States Patent [19]

Sisti et al.

[11] **4,405,344**

[45] **Sep. 20, 1983**

[54] **METHOD AND EQUIPMENT FOR VOLUMETRICALLY CONTROLLED AND REPRODUCIBLE INTRODUCTION OF SMALL AMOUNTS OF LIQUID SAMPLES INTO CHROMATOGRAPHIC ANALYSIS SYSTEMS**

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[73] Assignee: **Carlo Erba Strumentazione S.p.A.**, Rodano, Italy

[21] Appl. No.: **304,780**

[22] Filed: **Sep. 23, 1981**

[30] **Foreign Application Priority Data**

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May 4, 1981 [IT] Italy 21504 A/81

[51] **Int. Cl.**³ **B01D 15/08**
[52] **U.S. Cl.** **55/67**; 55/197; 55/386
[58] **Field of Search** 55/197, 67, 386

[56] **References Cited**

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Primary Examiner—John Adee
Attorney, Agent, or Firm—Millen & White

[57] **ABSTRACT**

A method and apparatus for controllably and reproducibly introducing small amounts of a liquid sample into chromatographic systems, especially high resolution gas chromatographic systems and thin-layer chromatographic systems, order to obtain sampling extremely reduced in volume and presenting maximum reliability and reproducibility, uses a sample container having a pipette-like or nozzle-like outlet neck of very small diameter. The liquid placed in the container is submitted to at least one pressure pulse which is controlled in time and/or amplitude in order to determine emission of a corresponding and controlled quantity of liquid from the outlet neck. The pressure pulse can be a pneumatic pulse directly applied to the liquid sample in the container, or a mechanical pulse applied to the liquid sample through a device acting by magnetostriction or by a piezoelectric system.

22 Claims, 9 Drawing Figures

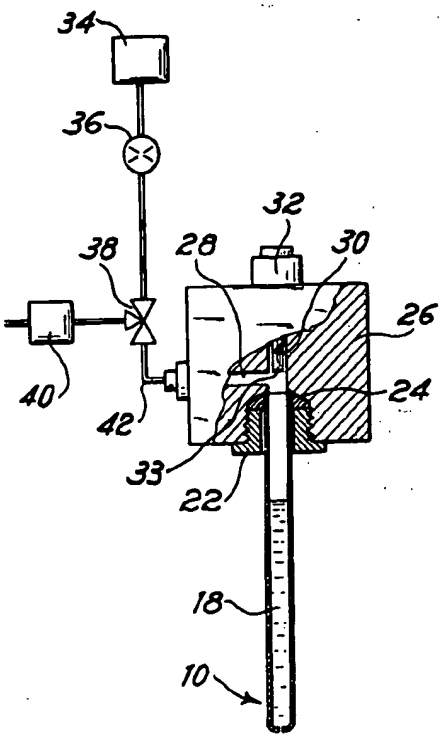


Fig. 1

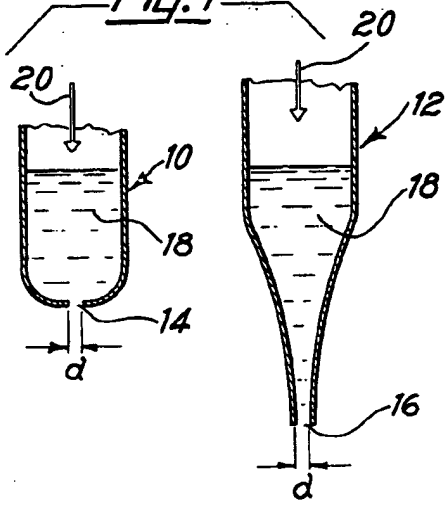


Fig. 2

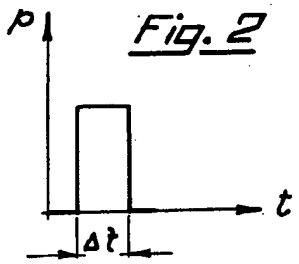


Fig. 3

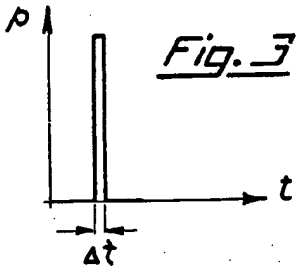


Fig. 4

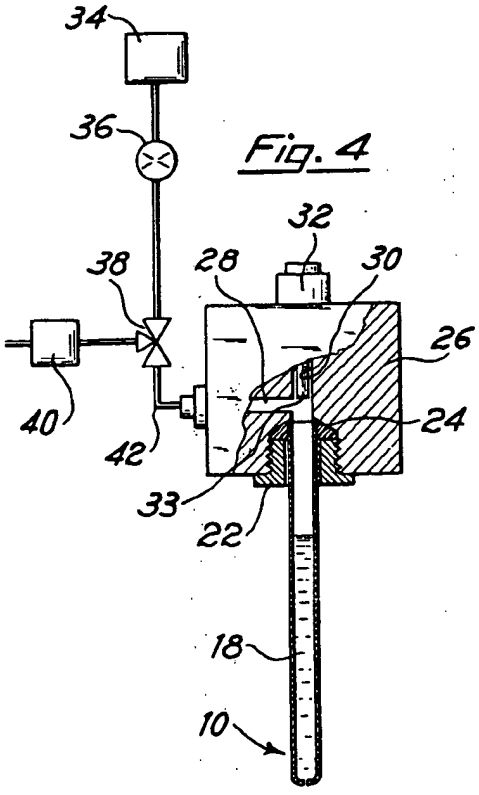
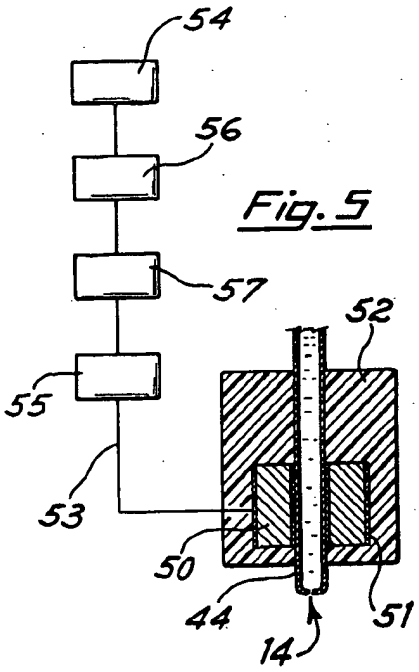


Fig. 5



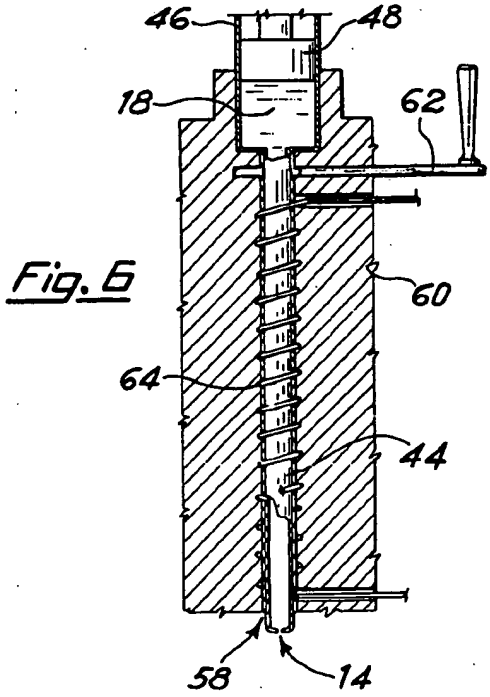


Fig. 6

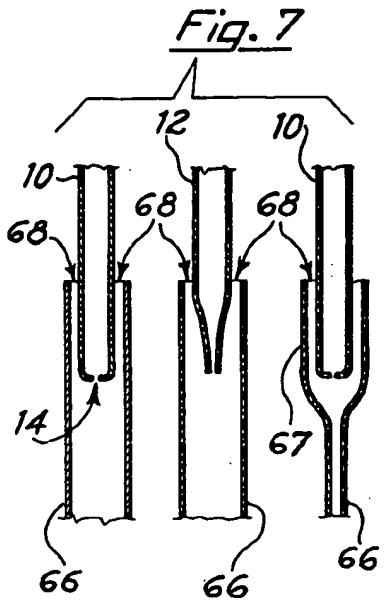


Fig. 7

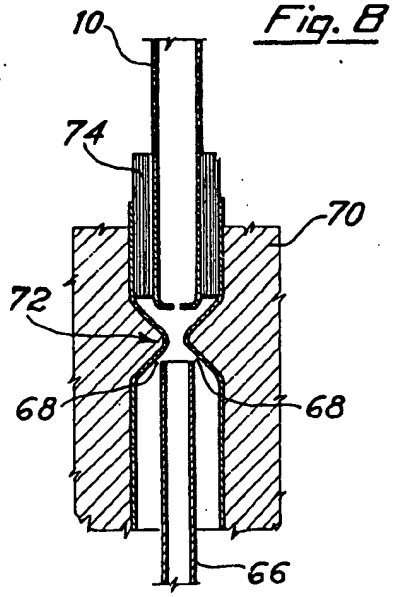


Fig. 8

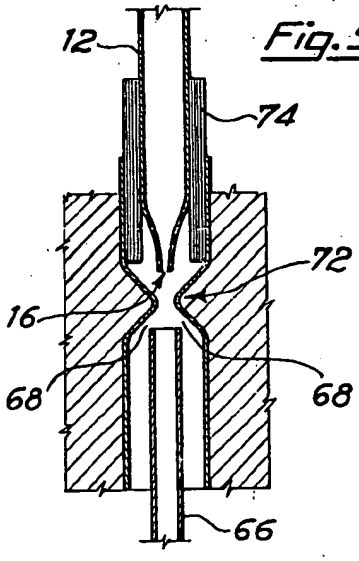


Fig. 9

METHOD AND EQUIPMENT FOR VOLUMETRICALLY CONTROLLED AND REPRODUCIBLE INTRODUCTION OF SMALL AMOUNTS OF LIQUID SAMPLES INTO CHROMATOGRAPHIC ANALYSIS SYSTEMS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a method and an apparatus to perform sampling in chromatographic systems with very small amounts of liquid sample, said method and said equipment being particularly but not exclusively applicable to high resolution gas chromatographic systems, with capillary or micropacked columns, or to thin-layer chromatographic systems. Use of this method and apparatus makes it possible to perform controllable and reproducible sampling of very small amounts of sample, with values unattainable through the techniques usually employed for liquid sampling in chromatographic systems and particularly using microsyringes or pipettes.

2. Description of the Prior Art

The microsyringes used in chromatography are generally of the type with calibrated body (capable of sampling amounts ranging from 0.2 to 10 microliters) or of the type with calibrated needle, where the piston penetrates into the needle. The latter microsyringes are capable of handling smaller quantities of samples, in a reliable and reproducible way, but only within certain limits, in particular with lower limits of about 200–300 nanoliters. Below this limit, the high surface tension of the liquid and the relatively reduced speed of the piston movement do not allow the drop, which has formed at the needle end, to fall from it, considering the reduced diameter of the outlet nozzle of the needle. Precision is moreover negatively affected by poor sealing between piston and calibrated needle.

Another known system is the sampling system commonly used in the laboratory and named "pipette system", in which a calibrated tubing is filled with a liquid to be transferred, by sucking it into the tubing, the liquid amount placed in the tubing is controlled and the same is retained in the tubing by closing same at the end where aspiration has been carried out. Then, an injection of the liquid into a receiving container is made by opening said end. This sampling method or transfer method of determined amounts of liquid is well known and has been used in gas chromatography too, but however only for quantities usually measurable in a rather rough way, even if the literature reports a lower limit of 25–50 nanoliters (see R. Kaiser-Gas Phase Chromatography—Vol. 1 pp. 90–95—Butterworths 1963—London).

Therefore it can be considered that, of course according to the nature of the liquid substance to be sampled, a lower limit exists, generally between 50 and 200 nanoliters, below which it is not possible to go in reliable and reproducible sampling using microsyringes or micropipettes.

Furthermore, in the case of the pipettes, and also often in the case of the microsyringes, when the gaseous fluid between the piston and the sample liquid is not completely eliminated, considering that a quantitative determination is performed during feeding, it may occur that the gas pushing the liquid out of the pipette, enters the capillary column, which sometimes involves problems connected with the choice and the use of said gas, such that it does not affect analyses.

The above mentioned quantitative limitations, however, are such that the operator is often forced to perform accessory operations imposed by the relatively high quantities of sample that has to be introduced into the chromatographic system and particularly into the capillary column. In fact, especially in the case of direct injection without vaporization (oncolumn), particularly considered herein, the sample must be diluted in a dilution ratio which is often very high (of the order of 1:10000 or more), with an operation which may involve difficulties in the exact analytical determination of the sample and in that it can introduce discriminations or variations in the sample original conditions.

In other cases, a co-called "splitting" operation is necessary, that means the elimination of a high percentage of the quantity fed to the injector, before its introduction into the column, which operation may involve even higher risks of discriminations.

OBJECTS OF THE INVENTION

Accordingly, an object of the invention is to provide a method and apparatus making it possible to perform sampling in chromatographic systems with very small amounts of liquid sample; for instance from 10 picoliters to 50 nanoliters, in a reproducible way under the same sampling conditions and sample type.

Another object of the present invention is to provide a method and apparatus in which the introduction of said small or very small amounts of liquid sample does not require the parallel introduction into the chromatographic system of another substance, either a liquid or gaseous one to propel the sample out of the sampling container.

Still another object of the present invention is to provide a method and apparatus wherein the volumetric dosage of the substance injected into the chromatographic system is performed with the maximum of reliability, precision and reproducibility, during the injection stage itself.

SUMMARY OF THE INVENTION

According to the invention a method is provided for injecting very small quantities of liquid sample in chromatographic systems, especially in high resolution gas chromatographic systems or thin layer chromatographic systems, said method comprising the following steps:

preparing a sample container having a volume larger than the quantity to be sampled and having a pipette- or nozzle-shaped neck, acting as outlet, with a maximum size ranging between 1 and 100 μm, preferably between 1 and 30 μm;

filling said container with a sample quantity greater than the amount to be sampled and controlling that, in correspondence with said outlet, a meniscus of the sample liquid be formed;

introducing said container, or part of same, into an injector or a device for sample injection into the chromatographic system;

submitting the liquid inside the container to at least one pressure pulse having a pressure value sufficient to overcome the surface tension of the liquid sample in correspondence with the outlet and a duration limited in time according to said pressure value and to the quantity to be sampled, keeping into consideration the outlet

size, the pressure pulse amplitude and the sample characteristics;

abruptly reducing the pulse pressure down to a value lower than the one sufficient to overcome the surface tension of the liquid in correspondence with the container outlet.

Therefore, according to said method, it is now possible to carry out sampling of extremely reduced quantities of liquid, using a container which can be of the substantially traditional type, except for the outlet neck, to which a device can be applied which allows to create said pressure pulse, in such a way that the sample drawing and eventual washing of the container can be performed with extremely simple and traditional systems and means, though being possible to perform said samplings with extremely reduced quantities.

Considering the performance of the system according to the invention, in most cases, the sample can be injected directly into the column, without vaporization. Vaporization may become necessary only in case of so-called "dirty" samples to prevent substances with high molecular weight contained in said samples from being directly introduced into the capillary column.

Said method according to the invention can be substantially performed according to two different ways, the first of which considers the application of a pneumatic pulse, obtained by operating for time periods of the order of milliseconds an electrovalve which makes it possible to exert a pressure in correspondence with a gaseous element directly or indirectly placed in contact with the sample in the container, while the second one considers the application, to the container body and/or to means mechanically connected with the same, of a mechanical pulse, obtained with a piezoelectric system, a magnetostrictive system or other similar system, which determines an extremely high and extremely quick pressure increase in the liquid.

As previously said, the invention relates also to an apparatus which, according to said method, makes it possible to perform samplings in chromatographic systems with small or very small quantities of liquid sample, in a reliable and reproducible way under the same operating conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an enlarged view, in axial section, showing possible shapes of the outlets of containers for samples to be used according to the invention.

FIGS. 2 and 3 are examples of possible pressure pulses used in the method and equipment according to the invention to perform desired sampling.

FIG. 4 is a diagrammatic view, with parts in section, of an apparatus for performing sampling by the use of pneumatic pulses.

FIGS. 5 and 6 diagrammatically show embodiments of apparatus for sampling by means of pressure pulses obtained with a piezoelectric system and magnetoscrictive system, respectively.

FIG. 7 is a diagrammatic view showing possible reciprocal positions of container outlets, in one of the types of apparatus previously illustrated, and the end of a capillary column in applications to high resolution gas chromatography with capillary columns.

FIGS. 8 and 9 are diagrammatic views of other possible positions of the sample container and of the end of the capillary column for the same uses as considered in FIG. 7.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates, as already said, to a method and an apparatus for sampling, in chromatographic systems, of liquid samples, said method and said apparatus being particularly applicable to laboratory chromatographic systems, especially in high resolution gas chromatography with capillary columns and with direct on-column injection of the sample, without previous vaporization, or to thin-layer chromatographic systems. In particular, chromatographic systems of the direct injection type are well known and applied, wherein the liquid sample is injected directly into the initial part of the column and then is moved along the latter by means of a carrier gas, the sample being simultaneously vaporized. The method and equipment according to the invention are capable, especially in chromatographic systems of the above mentioned type, of allowing injection of a quantity of liquid sample so reduced in volume that the entire quantity can be analyzed in the chromatographyc sistem, without any need of dilution, as is necessary in traditional methods and apparatus to obtain a precise dosage of the injected sample. Although larger amounts can also be injected, the method and apparatus which will be illustrated are particularly suitable for the injection in a reliable and reproducible way, of liquid samples in quantities ranging between 10 picoliters and 50 nanoliters, i.e., amounts so small that they could not be handled with the previously employed systems.

The method and the apparatus of the invention are based on the use of a container for the liquid sample which has a volume greater than that of the sample to be analyzed, said container being made of any suitable material, for instance glass, metal, fused silica or any other material, and having a pipette shape, a syringe needle shape or any other suitable shape.

The essential condition is that said container, as indicated by 10 or 12 in FIG. 1, presents a neck defining an outlet having a maximum size (a diameter d in this specific case) ranging from 1 to 100 μm, preferably between 1 and 30 μm. Said neck can be nozzle-shaped, as indicated by 14 in FIG. 1, and therefore has a preferred neck diameter d from 10 to 30 μm, or micropipette-shaped, as indicated by 16 in the same FIG. 1, with a preferred diameter d from 1 to 20 μm. The container, 10 or 12 as illustrated in FIG. 1, can be filled by the usual methods, for instance by means of a syringe, by gravity, by capillarity or in any way whatsoever, with a quantity of liquid 18 which is greater than the volume that is required to flow from the neck 14 or 16 to be analyzed in the chromatographic system. Once the container 10 or 12 has been fed with the sample 18, it is necessary to check, especially in the case of the container 12 with a pipette-like end, that the liquid goes as far as to reach the neck 14 or 16, forming a meniscus therein. Under these conditions, taking into account the reduced size of the neck, the surface tension of the liquid prevents the latter from flowing out of the neck, forming drops, this obviously provided that the neck 14 or 16 does not touch foreign bodies, as for instance the wall of the gas chromatographic column, which may help the liquid to flow outside the column. Once the feeding of the container 10 or 12 as previously indicated has been carried out, pressure on the liquid 18 is exerted directly or indirectly as schematically shown by the arrows 20 in FIG. 1 to eject a measured amount of liquid 18 through

the neck 14 or 16. Said pressure is controlled in amplitude and in time as indicated in FIGS. 2 and 3, keeping into consideration that the liquid amount ejected depends both on the amplitude, namely the value of the pulse pressure, on the duration of the pulse itself and on the number of pulses.

As it can be seen from FIGS. 2 and 3, the pulse has a step configuration and it is very important that the downwards section of same, namely pressure return to zero, be placed vertically as much as possible in order to avoid during this stage, in correspondence to the neck 14 or 16, the formation of a drop which will remain in position, thus completely altering any measuring of the injected sample. In the case of FIG. 2, the pressure pulse is a pneumatic pulse and reaches a value p_1 which must be in any case higher than that necessary to overcome the surface tension of the liquid in correspondence with the neck 14 or 16. In relation to the pressure value p_1, the duration Δt of the pulse is chosen, variable from about a millisecond to about a second, in order to obtain the desired quantities of ejected liquid.

The pressure values can range from 1 to 10 atmospheres. Alternatively, it can obsiously be possible to emit several pulses having lower duration. However, for an exact reproducibility of sampling, it is very important to reduce to the minimum the dead space, that is the gas volume on which each pressure pulse acts.

The situation illustrated in FIG. 3 is the one that occurs in case of a pressure pulse exerted by means of magnetostriction, or piezoelectricity. In this case, the time Δt is extremely reduced and corresponds to the resonance frequency of the laminations forming the magnetostriction device or to the frequency of the piezoelectric material, ranging from 10 to 100 KHZ, while a certain regulation of the quantity emitted at each pulse can be performed by varying the amplitude of the electric pulse given to the device and consequently the pulse pressure value given to the liquid, said pressure value being in any case of one or several orders higher than that necessary in the pneumatic case.

An apparatus for carrying out the method according to the invention with pneumatic pulse is diagrammatically illustrated in FIG. 4, wherein a receiver 10 of the type illustrated in the left side of FIG. 1 is provided, which has preferably a very small inner diameter and which can be removably fixed, for example by means of a locking nut 22 and a gasket 24 compressed by said nut, in a supporting block 26 which presents a groove 28 for feeding gas under pressure, for instance air. The block 26 can present another groove 30 which can be closed by a locking element 32 to feed the container 10 with the sample 18. The locking element comprises a protrusion 33, for example in metal, to reduce to the minimum the dead space.

The duct 28 is connected, in any known way, to a circuit for supply of air under pressure coming from a source 34, said circuit comprising a pressure regulator 36 and an electrovalve 38, the opening time of which can be regulated by means of a device 40, known in itself. A duct 42 is positioned between the electrovalve 38 and block 26 and advantageously presents a very small inner size, in such a way to reduce to the minimum the so-called dead volume, that is the volume of air comprised between the valve 38 and the upper meniscus of liquid 18 within the container 10. Said reduction of the dead volume is very important to obtain a quick fall of pressure at the end of the pulse and then to be sure that no drop forms and adheres to the container 10 outlet.

The apparatus of FIG. 4 can be fed through the opening 32 for instance by means of a conventional syringe, with a sample quantity much greater than the one which must be used for injection into the gas chromatographic system. Once the feeding is performed, the opening 32 is closed, the container is introduced into the chromatographic system and the valve 38 is opened for a time period controlled by the device 40, in such a way as to create a pressure pulse having a value regulated by the element 36. In this way, a small jet of liquid forms and breaks out in small drops at a point which is farther from the outlet neck 14 the higher is the pressure value set in regulator 36, of course with the same type of liquid and same size of neck.

The sampling device illustrated in FIG. 5 operates with a piezoelectric system which, in its that part including the element for formation and emission of the jet of drops, is substantially configured like an ink-drops printing device, of the "jet on demand" type, known in itself. It essentially comprises a container 44, for example but not necessarily with a cylindric shape, made of glass or fused silica, ready to be filled in its lower section with the sample to be injected into the column for instance introduced through the upper section thereof.

The container 44 ends in its lower section with a calibrated nozzle 14, as previously indicated, through which the liquid jet of sample is emitted. Under atmospheric pressure conditions, the liquid does not flow out of the nozzle 14 because of its surface tension.

The container 44 is connected to a transducer 50 of a piezoelectric type, capable of provoking, when excited, a sudden volume variation inside the container 44 and therefore a sudden pressure variation in the liquid present in it, such as to determine the flowing out of a calibrated jet of one or more drops through the nozzle 14. The transducer 50, which is placed very near to the nozzle 14 and housed for example in a small block 52 made of plastic material, is excited by means of a source of electrical pulses 54, which have the characteristics previously described, feeding of the transducer 50 on its electrodes 51, through a circuit 53 having a switch 55 by means of which the operator can close the circuit and then control the emission of the jet of drops. To the source 54 a known means 56 is connected to vary the amplitude of pulse or pulses communicated to the transducer 50; another component 57 is also connected, capable of controlling the duration of the pulse or series of pulses.

For operation, the container 44 must be filled with the liquid sample, possibly by means of a microsyringe, at least in its section close to the nozzle 14, and the transducer 50 is submitted to a pulse or series of pulses having a predetermined amplitude, during which, in correspondence with the nozzle 14, a jet of one or more drops of sample liquid is emitted, in a small pre-settable quantity. Said jet presents high directionality and an extremely limited increase in diameter, so that it can be directed into a column with one of the systems which will be considered, for example through the injection port of a direct injector, equipped with a "slice" type or rotative valve.

The amplitude of pulses may be regulated by the element 56, in order to obtain a corresponding regulation of the pressure increase provoked by said pulses and consequently of a first parameter affecting the operative conditions, in particular of the correct formation

of the jet of drops, considering the nozzle diameter and the nature of the treated liquid. When the other conditions are kept unchanged, the ejected quantity of a given sample, thanks to the formation of a pulse having a preset amplitude, is exactly definite and equal to a drop, and therefore the ejected quantity obtained by a series of pulses will depend only on the duration of the latter and therefore on the number of pulses forming such series, because the time period of each pulse is determined by the transducer characteristics.

The apparatus illustrated in FIG. **6** consists of a container **44** having a needle-syringe shape **46** with piston **48**. The needle **44** is introduced into a duct **58** inside the body of an injector **60**, of a type known in itself and provided with a valve **62**, said injector body being provided with a magnetostriction apparatus **64**, acting on the needle **44**, which is made of nickel. The exerted pressure is sufficient for determining a sample ejection, in the desired quantity, independently from the position of the syringe piston **48**, obviously provided that the liquid **18** fills the container **44** at least partly and as far as the outlet neck **14**. Both in the case of a pneumatic-type pulse and in the case of a mechanical-type pulse, such as a piezoelectric or magnetostrictive one, when the method and the apparatus according to the invention are applied to a high-resolution gas chromatographic system with capillary column, it is possible to have two cases of a reciprocal disposition of container **10** or **12** and capillary column **66**, the first one of these cases being indicated in FIG. **7**. According to said figure, the container **10** or **12** shows an outer diameter which is smaller than the inner diameter of the capillary column **66** or of an enlarged end **67** of same, and is therefore inserted into the injector as far as to penetrate with its end section into the inlet opening of the capillary column **66**. In this case, the emission of liquid sample under the stated conditions is only conditioned by the fact that the neck **14** does not touch any component and in particular the column **66** wall. Both during sample injection and after this operation, a current of carrier gas is present, which penetrates into the column through the hollow space existing between the column wall and container **10**, as indicated by arrows **68**.

On the contrary, the case in which the capillary column has an inner diameter smaller or equal to the outer diameter of container **10** or **12** is illustrated, for the two types of container, in FIGS. **8** and **9**. In this case, the injector body **70** shows a neck **72**, which advantageously has an axial length as small as possible and on the two sides of which there are positioned the column **66** and the container **10** or **12**, the latter being preferably housed in a protecting collar **74**, which provides for seating of said container **10** or **12** on the inner wall of the injector. In this case, it is advisable, as already said, (i) that the distance between the outlet neck **14** or **16** of container **10** or **12** and the column **66**, as measured in an axial direction, be as small as possible, for instance 10 mm maximum, (ii) that the injection pressure of the jet into the neck **14** or **16** be sufficient for maintaining the latter in such a condition as to give rise to a very small opening angle of the jet, and (iii) that the axial alignment of the neck **14** and column **66** be perfect.

It is particularly important in this case that, during injection, the carrier feeding, always according to arrows **68**, be discontinued to avoid having the carrier gas drag the outside part of the injected sample, especially if the latter contains easily vaporizable substances.

The system according to the invention, herein described as an example, has proved to be very useful in particular for capillary columns with very small diameters, according to the present trend in this field.

Finally, it should also be noticed that the embodiments of the present invention as above illustrated and described can be submitted to several changes and variations without departing from the spirit and scope of the invention itself, these variations comprising the use of an automatic sampler, wherein the container is automatically filled and wherein sample emission occurs through several jets of sample.

What is claimed is:

1. A method for reproducibly injecting a very small, volumetrically controlled liquid sample for chromatographic analysis, said method comprising the steps of:

(a) preparing a sample container having a larger volume than the desired sample volume and having a pipette- or nozzle-shaped neck at its outlet, terminating in an opening with a diameter of 1–100 μm;

(b) introducing into said container a volume of liquid sample greater than the desired sample volume, in such a manner that a meniscus of the sample liquid forms at said outlet opening, and positioning the filled sample container for sample injection into the chromatographic system; and

(c) applying to the liquid inside the sample container at least one pressure pulse of controlled duration and amplitude, the maximum amplitude of each said pressure pulse being higher than the pressure necessary to overcome the surface tension of the sample liquid at said meniscus, the duration being sufficient to eject a desired volume of sample liquid, and the pressure drop at the end of each pulse being sufficiently abrupt as to avoid formation of a droplet of sample which is not ejected but instead adheres to said outlet opening.

2. A method according to claim **1**, wherein said sample container further comprises a zone of small volume, opposite to the outlet opening and usually not filled with liquid, said zone communicating with a source of gas under regulatable pressure, through a valve whose opening time is controllable, and a duct, said duct having a small volume between the valve and the container; and wherein said pressure pulse is controlled by regulating said gas pressure and controlling the opening time of said valve.

3. A method according to claim **2**, wherein said gas pressure is from 1 to 10 atmospheres, and said valve opening time is from 1 millisecond to 1 second.

4. A method according to claim **1**, wherein each pressure pulse is produced by magnetostriction or by piezoelectric means.

5. A method according to claim **4**, wherein at least part of said sample container, in the portion thereof containing the liquid sample, cooperates with a piezoelectric transducer to and wherein said transducer is submitted to at least one electric pulse to give rise to a corresponding pressure pulse in the liquid inside the container.

6. A method according to claim **4**, wherein at least part of said container, in the portion thereof containing the liquid sample, is made of nickel; wherein a control device with magnetostrictive actuation cooperates with the nickel part of said container, when the latter is in operative position; and wherein at least one current pulse having an adjustable amplitude is applied using said control device to give rise to a corresponding pres-

sure pulse in the liquid contained in the nickel part of the container.

7. A method according to claim 1, wherein the diameter of the sample container outlet opening is 1-30 μm.

8. A method according to claim 1, wherein the amount of sample injected is 10 picoliters-50 nanoliters.

9. A method according to claim 8, wherein the entire sample is injected directly into a capillary column in a high-resolution gas chromatographic system, without any sample splitting.

10. An apparatus for volumetrically controlled and reproducible injection of small quantities of liquid sample directly into a capillary column of a high-resolution gas chromatographic system without use of a sample splitter, comprising, in combination with an on-column sample injection device for a high-resolution gas chromatographic system:

a sample container having a volume greater than a desired liquid sample volume and having a pipette- or nozzle-shaped neck at its outlet, terminating in an opening with a diameter of 1-100 μm;

positioning means for operatively positioning said sample container in said on-column sample injection device, for injection of a sample through said outlet opening and directly into a capillary column; and

pulse means communicating with said container for applying to a liquid inside the container at least one pressure pulse of controlled amplitude and duration, and ending in an abrupt pressure drop.

11. An apparatus according to claim 10, further comprising a source of gas under pressure and means for regulating its pressure; connecting means for pneumatically connecting said gas source to the sample container on the side thereof opposite to the outlet opening, said connecting means comprising a valve, means to control its opening time, and at least one duct connecting said valve to the sample container, said duct being sized in such a way as to minimize the gaseous volume between liquid in the sample container and the control valve.

12. An apparatus according to claim 11, wherein said pressure regulating means is capable of adjusting the gas pressure to a value between 1 and 10 atmospheres and said valve control means is capable of controlling the opening time of the valve to a time interval between 1 millisecond and 1 second.

13. An apparatus according to claim 10, wherein said pulse means comprises at least one transducer capable of producing at least one pressure pulse of high amplitude and short duration in the sample container; a pulse source for exciting said transducers; and means for adjusting at least one of the amplitude or the number of the pulses.

14. An apparatus according to claim 13, wherein said transducer is a piezoelectric tranducer, and said pulse source is a source of electric pulses of high amplitude and steep drop-off.

15. An apparatus according to claim 14, wherein said sample container is made of glass or fused silica and is surrounded, near to the outlet opening thereof, by a piezoelectric transducer embedded in a small block of a plastic material.

16. An apparatus according to claim 13, wherein the sample container has at least a part thereof made of nickel in the portion containing the liquid sample; wherein the transducer comprises a magnetostrictive device capable of acting on said container part when in its operative position within a seat provided in said magnetostrictive device, and actuating means for applying to said magnetostrictive device at least one current pulse having an adjustable amplitude, thereby producing a corresponding pressure pulse acting on the liquid contained in the nickel part of said container.

17. An apparatus according to claim 16, wherein said pulse has a duration which depends on the frequency of mechanical resonance of the magnetostrictive device and is adjustable in amplitude.

18. An apparatus according to claim 10, further in combination with a capillary column; wherein said sample container has at least an end section having a maximum size smaller than the inner diameter of said capillary column where chromatographic separation occurs; and wherein said positioning means cooperates with said on-column injection device for axially controlling the sample container position so that its end section penetrates into the column inlet opening without touching the walls thereof.

19. An apparatus according to claim 10, further in combination with a capillary column; wherein said capillary column has an inner diameter smaller than the neck of said sample container; and wherein said positioning means cooperates with said injection device for reciprocally aligning and axially centering the chromatographic column inlet end and the sample container outlet end in an axially spaced position at a distance not exceeding 10 mm.

20. An apparatus according to claim 19, wherein said chromatographic system further comprises means to discontinue carrier gas feeding during injection.

21. An apparatus according to claim 19 which further comprises a protecting collar for seating the sample container outlet end on the inner wall of the injector.

22. An apparatus according to claim 10, wherein the diameter of the sample container outlet opening is 1-30 μm.

* * * * *

United States Patent [19]

Gilak

[11] Patent Number: 4,832,916

[45] Date of Patent: May 23, 1989

[54] CHROMATOGRAPHIC COLUMN FOR IMMUNOLOGICAL DETERMINING METHODS

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[21] Appl. No.: 18,085

[22] Filed: Feb. 24, 1987

[30] Foreign Application Priority Data

Mar. 17, 1986 [DE] Fed. Rep. of Germany 3608883

[51] Int. Cl.[4] G01N 30/02; G01N 30/48; G01N 33/538

[52] U.S. Cl. 422/70; 210/656; 210/658; 436/541; 436/807; 436/810

[58] Field of Search 436/541, 807, 810; 422/70; 210/656, 658

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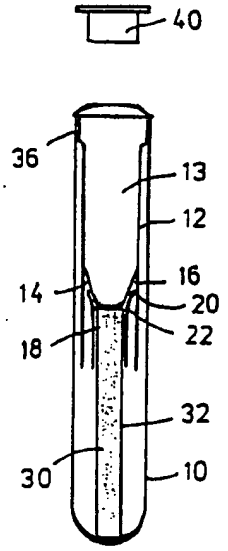
Primary Examiner—Sidney Marantz
Attorney, Agent, or Firm—Balogh, Osann, Kramer, Dvorak, Genova & Traub

[57] ABSTRACT

The invention relates to a chromatographic column for separating antigen-antibody complexes from the free antigens not bonded to the antibodies and/or for complete bonding of all marked substances in the immunological determination of antigens or haptens by radioimmunological, luminescence-immunological, fluorescence-immunological or enzyme-immunological determination methods in which a crepe-like tightly rolled filter paper of high degree of purity, in particular of regenerate cellulose, is pressed into a water-proof and water-tight envelope as nonpolar column material. According to the invention the chromatographic upright column comprises an upper reaction portion chargeable from above and a lower separation and measuring portion which are separated from each other by a perforable bottom, the column being fastened from below on the perforable bottom of the column.

10 Claims, 2 Drawing Sheets



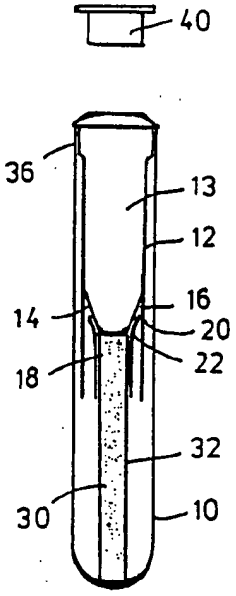


FIG. 1

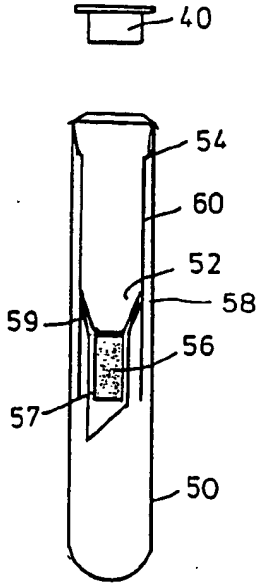


FIG. 2

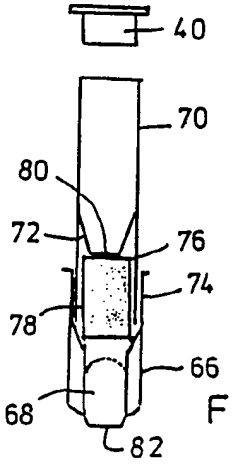


FIG. 3

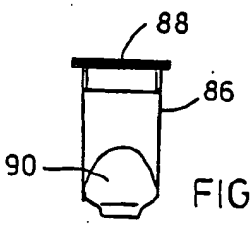


FIG. 4

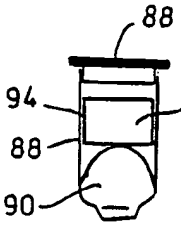


FIG. 5

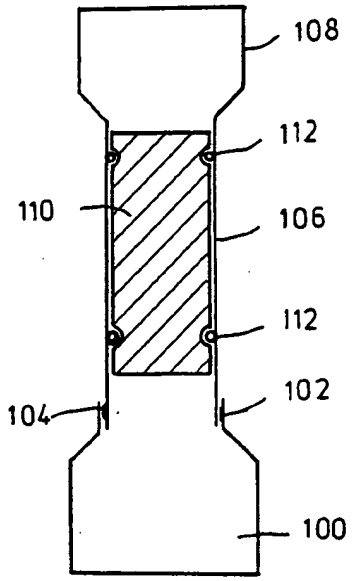


FIG. 6

CHROMATOGRAPHIC COLUMN FOR IMMUNOLOGICAL DETERMINING METHODS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to a chromatographic column for immunological investigation methods.

Such a chromatographic column is intended for separation methods for immunological determinations, in particular fluorescence-immunological, enzyme-immunological or luminescence-immunological investigations, in which the antigen-antibody complex form is separated from the free antigens not bonded to the antibody with the aid of the dry chromatographic column to which the liquid reaction mixture of the immunological determination is introduced, the antigen-antibody complex solution being measured extinction-photometrically.

SUMMARY OF THE INVENTION

The invention has as its object the provision of a particularly economical chromatographic column, easy to handle, for such investigation methods which permits in particularly simple and reliable manner separation and measurement of the substances to be investigated.

This objective is achieved according to the invention in a dry chromatographic column for separating antigen-antibody complexes from the free antigens to bonded to the antibodies and/or for complete bonding of all marked substances in the immunological determination of antigens or haptens by radioimmunological, luminescence-immunological, fluorescence-immunological or enzyme-immunological determination methods in which a crepe-like tightly rolled filter paper of high degree of purity, in particular of regenerate cellulose, is pressed into a water-proof and water-tight envelope as nonpolar column material by the improvement that the chromatographic upright column of filter paper comprises an upper reaction portion chargeable from above and a lower separation and measuring portion which are separated from each other by a perforable bottom, the column being fastened from below on the perforable bottom of the column.

It is achieved with the invention that reaction on the one hand and separation and measurement on the other can be carried out in a single column, possibly without outer contact, and the material to be investigated, in particular the radioactive material, remains completely in the filter and can be measured in extremely simple manner by means of a gamma counter in the case of radio-immunological investigations or photometrically in the case of luminescence-immunological investigations.

An important desired secondary effect in the radio-immunological determinations with the column according to the invention is that in radio-immunological determinations the entire radioactive reaction mixture remains absorbed in the column. After the counting of the sample the radioactivity is thus in a form which is easy to handle; the danger of contamination with residues of the liquid reaction mixture is greatly reduced compared with known methods. Even when the antibody is stationarily bonded to a substrate substance within the test tube the entire liquid radioactivity is absorbed in the column; at the same time the free antigens are bonded in the upper column portion.

Preferably the filter paper column is attached from below tightly to the perforable bottom of the reaction portion chargeable from above. In the perforation a particularly effective penetration and likewise adsorption is achieved on perforation up to the depth of the filter paper. Preferably, the filter paper column can have at the top a funnel-shaped widening with which it is attached in clamping fit beneath the similarly shaped bottom of the column.

The filter paper may be pressed into a plastic sheath which may possibly be surrounded by a metallic sheath, in particular of galvanized copper. The latter is suitable as screening material in the case of extinction-photometric or radio-immunological measurements.

The perforation may also be made from the outside. It is however also possible to equip parts in contact with the bottom with tines, prongs or the like which for instance under pressure on the two ends of the column perforate the bottom (closed system). A particular use of column and filter in automatic devices is also possible with the invention. In this case two tubular microcuvettes closed at the bottom are disposed adjacent each other, the first serving as reaction vessel with perforated cover. The second serves as separating and measuring vessel and contains in the upper portion the column-like filter paper tightly fitted into a plastic sheath and in the lower portion the extinction-photometrical measurement is carried out. Instead of using two microcuvettes a single device is also possible, the reaction portion described with perforable bottom being disposed at the top and the separating and measuring portion at the bottom. The separating portion consists of the filter paper column described above which is widened in funnel-shaped manner at the top and attached in clamping fit beneath the bottom of the reaction portion. The measuring portion is a microcuvette which is also pushed from below over the plastic sheath or sleeve of the reaction portion.

In this embodiment it is possible to lyophilize the reagents, except for the substance to be determined, at low temperatures, in particular beneath −20° C.

Finally, it is possible when using the step according to the invention in automated devices to make all the columns perforable employing a single charging operation, manually or automatically. The charging of the column is from above as in all further developments of the invention.

A particularly expedient important further development of the invention resides in that the crepe-like filter material to increase its bonding affinity is impregnated with an organic acid, in particular maleic or oxalic acid or possibly a base, and the impregnating agent washed out again. This imparts to the paper when dried again an improved absorbability and differentiation of the absorption due to a certain swivelling of the fibres.

BRIEF DESCRIPTION OF THE DRAWINGS

Examples of embodiment of the invention will be described in more detail hereinafter with the aid of the accompanying drawings, wherein:

FIG. 1 shows a chromatograhic column for the radio-immunological investigation method (radio-immuno-assay:RIA);

FIG. 2 shows a chromatographic column for example for fluorescence-immunological or luminescence-immunological investigation methods;

FIG. 3 shows an embodiment with microcuvette;

FIGS. 4/5 shows reaction vessel and measuring vessel for automated devices and

FIG. 6 shows a further embodiment.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

FIG. 1 shows for radio-immunological determinations a test tube 10 into which an upright chromatographic column 12 is inserted in tight or sealing manner and comprises substantially in the centre a bottom 14. The column with the exception of the filter paper and the test tube consists of a transparent plastic, preferably polyethylene. The bottom 14 of the reaction vessel is attached firmly and sealed with respect to the column. The bottom 14 is made conical or funnel-shaped towards the bottom at 16 and comprises a thin perforable base 18.

The conicity of the funnel bottom results in a corresponding annular space 20 which is formed from the inner wall of the straight column tube and the outer wall of the funnel-like bottom and tapers to a point at the top. This simple construction is utilized to permit a clamping connection in the manner to be described below.

Complementary to this funnel bottom the filter and measuring portion of the separating vessel comprises a sleeve or sheath which merges into a complementarily funnel-shaped widened portion 22. The sheath may consist of metal but also of plastic. Tightly pressed into the sheath is a filter 30 of prepared crepe paper which forms the actual column material. It comprises an envelope 32 of an outer stiff plastic. The column material may consist of cellulose pretreated with chemical impregnating agents, for example with organic acids, preferably maleic or oxalic acid or possibly with a base. The reaction vessel provided with sheath is inserted into the funnel extension of the column in such a manner that the filter 30 contains the perforable bottom.

In the execution of the sample determination according to the invention the reagents are pipetted into the reaction vessel 13. After setting the reaction equilibrium at the end of the incubation time, with a pointed object the funnel-shaped bottom of the reaction portion is perforated from below or preferably from above, this being done a considerable distance into the crepe filter. The liquid reaction mixture runs through the perforated bottom into the column-shaped filter. When this is done separation takes place in that the unbonded phase, i.e. the free antigens not bonded to antibodies, is absorbed in the upper column portion whilst the bonded phase, i.e. the antigen/antibody complex, migrates into the lower column portion. All the radioactive liquid is absorbed by the filter paper enclosed in water-tight manner and can thus be easily dispensed without any danger of contamination. Of course, in the test execution the tube may be closed with a cover 40. Due to the close bearing of the filter material on the bottom of the reaction vessel and the continuous perforation excellent wetting of the entire column material 30 is ensured. Other possibilities of the vertical fixing of the filter paper with sheath within the entire column are of course possible, such as small ribs on the one part, recesses on the other part, an annular bead on the funnel-shaped bottom and an annular groove on the widened sheath, etc. It should be added that the upper column portion, the reaction vessel 13, is fitted via a widened portion 36 (fitting collar) into the sheath tube 10.

FIG. 2 shows an example of embodiment, particular for luminescence-immunological, fluorescence-immunological and enzyme-immunological investigation methods. In a sheath tube 50 a similar construction as in FIG. 1 in the form of a column with column head 54 fitting closely against the sheath tube inner side is inserted.

Whereas in the design of FIG. 1 the sleeve widening of the long filter is substantially in metal over a relatively short section, for the luminescence method only a short piece of filter 56, possibly with plastic outer sheath, is held in for example a long metallic sheath tube 57. A widened sleeve 59 consisting for example of plastic is attached in a similar manner to FIG. 1 over the complementary funnel-shaped bottom 58 of the column. However, in this case the widened sheath/shield 59 extends right to the top at the intermediate or annular space formed between the inner side of the straight column tube and the outer side of the funnel-shaped bottom 58 and is contained snuggly fitted in the latter on slight application pressure. The possibly metallic sheath tube 57 consisting of galvanized copper for locating the filter serves as shield. In the illustration the column is cut inclined at the bottom to ensure in constrained manner a dripping of the liquid possibly present only in small quantities. Embodiments without a shield 59 are also possible. The filter paper can also be pressed into the capillary sheath. For fluorescence-immunological investigation the reagents are pipette into the reaction portion 60 of the chromatographic column, including the marking substance. After setting the reaction equilibrium the funnel-shaped bottom of the reaction portion is perforated up to the filter paper lying closely therebelow and the liquid reaction mixture flows from above into the filter column. When this is done the components are separated in that the free phase is bonded i the upper column portion and the bonded phase, i.e. the antigen/antibody/complex to be measured, passes through the separating column and drips down onto the bottom of the test tube where it can thus be measured by extinction photometry. The shield 59 can be coloured. FIG. 3 shows an embodiment comprising a measuring cuvette 66 which is formed as microcuvette and in order to be usable for very small amounts of filtered liquid has a constriction in the lower region 68. Of interest is that the same column 70 with practically the same funnel-shaped bottom 72 as described with regard to FIGS. 1 and 2 fits into said measuring cuvette and is simply inserted with its lower open end 74. In this case the filter material 76 is pressed into a plastic sheath 78 which bears closely on the perforable bottom 80 of the funnel-shaped reaction vessel. The reaction vessel can once again be sealed by a cover 40. As in FIG. 1 the lower end of the sheath tube, in this case the microcuvette forms the measuring vessel and the column 70 the reaction and separating vessel. The antigen/antibody/complex can again be measured photometrically. In this case no separate shield is provided but this can be done. The microcuvette is a design with particularly small bottom 82 for measuring technical reasons.

The microcuvettes 86 and 88 of FIGS. 4 and 5 are particularly suitable for automated apparatuses. The microcuvette 86 forms the reaction vessel and the microcuvette 88 of FIG. 5 the measuring vessel.

The microcuvettes may for example be arranged grouped in racks. In the measurement the reaction vessel is then omitted and the measurement carried out in

the measuring vessel only. The two vessels are closed by tightly selling attachable covers **88**. The microcuvettes are again constricted at the bottom, firstly to fit properly into the holder of the automatic device and secondly to enable even very small defined volumes to be measured, as can be seen at **90**. In specimen investigation by immunological methods, in particular in fluorescence-immunological determinations, pipetting of the reagents into the microcuvette **86** as reaction vessel is carried out in a specific sequence. At the end of the incubation time the reaction mixture is aspirated and transferred to the filter in the measuring cuvette **88**. When the liquid passes through the column-like filter the components are separated as described with reference to FIGS. **2** or **3**. The liquid on the bottom of the measuring cuvette contains the antigen/antibody/complex and can be photometrically measured. The microcuvette **88** can have the same for as the microcuvette **86** of FIG. **4**. The filter may be of the same material as already described and explained in further detail below. The filter material here is placed in a sheath **94** consisting for example of plastic and completely filling the internal periphery of the microcuvette **88**.

Thus, according to the invention the reagent is introduced from above into the chromatographic column described, filtered and separated in the centre portion and the measurement is carried out at the bottom. The column material is a dry adsorption agent of nonpolar material in the form of a homogeneous felted crepe-like filter paper of high degree of purity rolled to form a tight column. The filter paper consists of pure linters having a polymerisation degree of 2000–3000 or of regenerate cellulose having a polymerisation degree of 800–3000 and is free of soluble substances. The filter paper has a uniform texture with bores of the order of magnitude of 1–14 μm and has a uniform absorbability over the height of the column. To increase the bonding affinity the filter paper is treated with acids, preferably maleic or oxalic acid, and possibly also with bases. The separation is carried out rapidly and extremely precisely by the cooperation of gravity and capillary forces. The separation takes place surprisingly in such a manner that the low-molecular components (free antigens and antibodies) are bonded i the upper column portion, i.e. after a short running time, whilst the high molecular antigen-/antibody/complexes migrate through the column unbonded. In FIG. **1**, with a column sealed at the bottom, the complexes remain in the lower column portion. In FIG. **2** with the column open at the bottom the complex is contained in the liquid dripping down at the bottom. Presumably, with the nonpolar adsorption agent van der Waals' and hydrophobic interactions predominate by which the migration behaviour can be explained.

In all the embodiments all reagents can be lyophilized in the column except for the substance to be determined.

The embodiments of FIG. **1** for radio-immunological determination includes a metal shield in the upper filter portion so that only the radioactivity of the antigen/antibody/complex disposed at the bottom is measured. Similarly, the embodiments for fluorescence-immunological or luminescence-immunological investigations (FIGS. **2**, **3** and **5**) include a shield of plastic round the column-like filter paper to avoid any light reflection.

Another possibility of carrying out a method according to the invention (FIG. **6**) is to use a reaction vessel **100** of a material as mentioned above possibly after fitting a cap which is not illustrated. After the reaction in the vessel **100** an addition of the marking substances the filter and separating portion **106** is attached tightly to the neck **102** of the reaction vessel. Seals **104** are indicated for this purpose. However, any other form-locking connection providing good sealing may be employed. A filter **110** of the type described above is tightly pressed into the separating and measuring portion **106**. The pressing action may be increase by ribs **112** which are only indicated and which can be provided at the top and bottom. The measuring and separating portion is continuous, i.e. the previously mentioned perforable bottom is omitted. After fitting on the entire unit is turned through 180° (turned upside down). The solution runs through the filter prepared in the aforementioned manner. The measurement is as described above. The separation of head and bottom portion can of course be at another point, for example near the lower section of the reaction vessel **108** or at any other convenient point.

The apparatus is preferably made available as kit, containing the chromatographic column and the usual known reagents for a specific test method, possibly in measured unit amounts.

The measuring apparatus can be formed again like the microcuvette of FIG. **5** (without bottom to be perforated) and made so that it can be connected in plug-type manner to the reaction vessel.

Finally, it is also possible, for example for automated apparatuses, to perforate a whole series of columns by pressing them on for example simulaneously. This may for instance be done by hand or via a plate. For this purpose for example the cover can be provided with long pointed prongs. The perforation is done by applying pressure to the cover. It would however be necessary in this case to make the "column" adequately stiff. The funnel-shaped bottom of the column is made substantially pre-weakened.

I claim:

1. Chromatographic column comprising means for separating antigen-antibody complexes from the free antigens not bonded to the antibodies and/or for complete bonding of all marked substance in the immunological determination of antigens or haptens by radio-immunological, luminescence-immunological, fluoresce-immunological. or enzyme-immunological determination methods, said chromatographic column being inserted in a sealing manner in a test tube and comprising an upper potion defining a reaction vessel for receiving reagents, a tightly roller filter material of high degree purity, an envelope made from water-proof and water-tight material tightly encompassing said filter material, said envelope encompassed filter material being secured in the lower portion of said column, said lower portion of said column defining a separation and measuring portion, and a perforable bottom member separating said reaction vessel from said encompassed filter material, whereby upon perforation of the bottom member, the reagents in the reaction vessel will flow through the filter material.

2. Chromatographic column according to claim **1**, wherein the filter material is pressed into a plastic sheath which is surrounded by a metallic sheath, in particular of galvanized copper.

3. Chromatographic column according to claim **1**, including a fitting collar at the upper end of the reaction vessel for inserting said column into a plastic test tube.

4. Chromatographic column according to claim **1**, wherein the filter material portion of the column to

increase its bonding affinity is prepared by chemical impregnating agents such as organic acids, in particular maleic acid or oxalic acid or possibly a base.

5. Chromatographic column according to claim 1, comprising a lower reaction vessel with attachable filter and measuring portion in which, tightly pressed crepe filter material is disposed which tightly closes the wall and is disposed in a sheath to increase the bonding affinity of said filter material, the column being tunable through 180° and placed upside down for the separating and measuring operation.

6. Chromatographic column according to claim 1, wherein two tube-like microcuvettes closed at the bottom are disposed adjacent each other, and as reaction vessel a column top is used or formation of the microcuvette itself as reaction vessel with performable cover, and on the second microcuvette a separating or measuring column top is disposed with a column of filter paper or insertion of the filter only into the second microcuvette, luminescence measurements taking place in each case solely at the second microcuvette.

7. Chromatographic column according to claim 1, wherein the filter material comprises a regenerated cellulose.

8. Chromatographic column according to claim 1, wherein the upper portion of the envelope carries at the top and outside a funnel-like widening with which it is fastened in a clamping fit beneath the bottom member material is pressed into a plastic sheath which is surrounded by a metallic sheath, in particular of galvanized copper.

9. Chromatographic column according to claim 8, wherein the filter material portion of the column is surrounded by plastic, as an adapting member to a column inner wall with which it is clamped against a conical-shaped bottom of the reaction vessel.

10. Chromatographic column according to claim 9, wherein the filter portion of the column is shielded from the bottom of the reaction portion in particular for photometric measurements, a conical-like widened shield consisting of a perforable bottom conical-shaped towards the bottom.

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US005882521A

United States Patent [19]

Bouvier et al.

[11] Patent Number: 5,882,521

[45] Date of Patent: *Mar. 16, 1999

[54] **WATER-WETTABLE CHROMATOGRAPHIC MEDIA FOR SOLID PHASE EXTRACTION**

[75] Inventors: **Edouard S. P. Bouvier**, Stow; **Randy E. Meirowitz**, Somerville; **Patrick D. McDonald**, Holliston, all of Mass.

[73] Assignee: **Waters Investment Ltd.**, Wilmington, Del.

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: **634,710**

[22] Filed: **Apr. 18, 1996**

[51] **Int. Cl.**[6] **B01D 15/08**

[52] **U.S. Cl.** **210/635**; 210/634; 210/656; 210/198.2; 210/502.1

[58] **Field of Search** 210/634, 635, 210/656, 659, 198.2, 502.1

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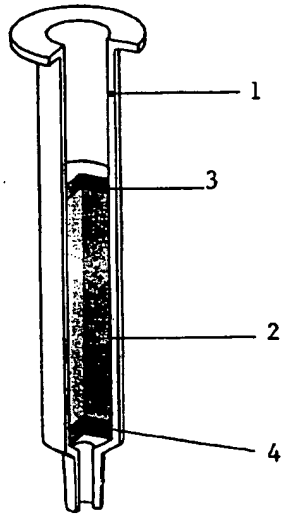
Primary Examiner—Ernest G. Therkorn

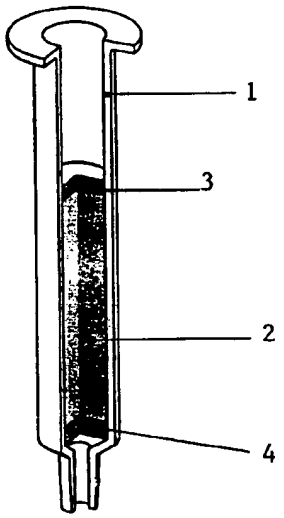
Attorney, Agent, or Firm—Hamilton, Brook, Smith & Reynolds, P.C.

[57] **ABSTRACT**

A method for removing an organic solute from a solution, comprising contacting the solution with a polymer formed by copolymerizing one or more hydrophobic monomers and one or more hydrophilic monomers, whereby the solute is adsorbed onto the polymer. The solution can comprise a polar solvent such as a polar organic solvent or water or an aqueous buffer. The hydrophobic monomer can be, for example, divinylbenzene. The hydrophilic monomer can be, for example, a heterocyclic monomer, such as a vinylpyridine or N-vinylpyrrolidone.

31 Claims, 1 Drawing Sheet

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WATER-WETTABLE CHROMATOGRAPHIC MEDIA FOR SOLID PHASE EXTRACTION

BACKGROUND OF THE INVENTION

Solid phase extraction is a chromatographic technique of frequent use in the preparation of samples for quantitative analysis, for example, via high performance liquid chromatography (HPLC) or gas chromatography (GC) (McDonald and Bouvier, eds. *Solid Phase Extraction Applications Guide and Bibliography*, sixth edition, Milford, Mass.: Waters (1995)). Solid phase extraction can be used to separate a component of interest in a complex solution from potentially interfering matrix elements and to concentrate the analyte to levels amenable to detection and measurement. Thus, solid phase extraction is of use in the analysis of environmental samples, where, for example, various soluble components of soils may interfere with the analysis of trace organic materials. Solid phase extraction is also of importance in the analysis of pharmaceutical agents or metabolites in blood plasma, which requires the prior removal of plasma proteins and other matrix constituents which may interfere with the analysis.

Solid phase extraction of an aqueous solution is typically performed by passing the solution through a single-use cartridge containing a chromatographic sorbent. The most commonly used sorbents consist of porous silica particles that have been functionalized on their surface with hydrophobic octyl (C_8) and octadecyl (C_{18}) functional groups. Prior to use, such sorbents must be wetted with a water-miscible polar organic solvent to solvate the alkyl chains. This increases the contact of these chains with the aqueous phase, increasing the sorbent surface area available to solutes and, therefore, retention of solutes. Such sorbents which are not pre-wetted or have dried out display poor solute retention, and, thus, inadequate separation of solution components.

The requirement that the sorbent remain wetted during the extraction procedure complicates solid phase extractions and substantially slows sample analysis. For example, solid phase extraction cartridges, in general, have differing flow rates and must be monitored individually to prevent drying out when used on a vacuum manifold, the current state of the art for processing multiple samples. This further complicates the development of instruments for automated solid phase extraction, which often incorporate elaborate safeguards to prevent drying out of the sorbent.

Thus, there is need for a solid phase extraction method which utilizes a sorbent that does not require wetting with an organic solvent or that stays wetted even if the bulk of the wetting solvent is removed during use on a vacuum manifold. Such a method would enable more rapid sample preparation for quantitative analysis, particularly for multiple samples, and allow the development of less expensive and simpler methods for automated solid phase extraction.

SUMMARY OF THE INVENTION

The present invention relates to a method for removing an organic solute from a solution. The method comprises contacting the solution with a water-wettable polymer formed by copolymerizing one or more hydrophobic monomers and one or more hydrophilic monomers, whereby the solute is adsorbed onto the polymer. The solution can comprise a polar solvent such as a polar organic solvent, a water/organic mixture or, preferably, water or an aqueous solution, such as an aqueous buffer, acid, base or salt solution.

The hydrophobic monomer can comprise a hydrophobic moiety. Suitable hydrophobic moieties include, but are not limited to phenyl, phenylene and C_2–C_8-alkyl groups. Suitable hydrophobic monomers include divinylbenzene and styrene.

The hydrophilic monomer can comprise a hydrophilic moiety. In one embodiment the hydrophilic moiety is a saturated, unsaturated or aromatic heterocyclic group, such as a pyrrolidonyl group or a pyridyl group. In another embodiment, the hydrophilic moiety is an ether group. Suitable hydrophilic monomers are, for example, N-vinylpyrrolidone, 2-vinylpyridine, 3-vinylpyridine, 4-vinylpyridine and ethylene oxide.

In one embodiment of the method, the polymer is a poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer which comprises greater than about 12 mole percent N-vinylpyrrolidone. In a preferred embodiment, the copolymer comprises from about 15 mole percent to about 30 mole percent N-vinylpyrrolidone.

The present invention further includes a method for forming a solution, containing a solute, which is suitable for quantitative analysis. In one embodiment, the method comprises contacting a first solution including the solute with a water-wettable polymer formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer, whereby the solute is adsorbed onto the polymer. This is followed by washing the polymer with a suitable solvent or mixture of solvents, so that the solute is desorbed from the polymer, thereby forming a second solution including the solute. This second solution is suitable for quantitative analysis.

In another embodiment, the invention provides a method for forming a solution comprising a polar organic solute which is suitable for quantitative analysis. The method comprises contacting a solution which includes the polar organic solute and at least one additional solute of lesser polarity with a water-wettable polymer formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer, whereby the additional solute is adsorbed onto the polymer and the polar solute remains in the aqueous phase. The resulting aqueous phase is, thus, a solution of the polar organic solute which is suitable for quantitative analysis.

The present invention further includes a solid phase extraction cartridge comprising an open-ended container and a polymer packed within the container. The solid phase extraction cartridge can, optionally, further comprise a porous retaining means, such as a frit. The polymer is formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer. Suitable polymers include poly(divinylbenzene-co-N-vinylpyrrolidone) copolymers which comprise about 12 mole percent or more, preferably from about 15 mole percent to about 30 mole percent, N-vinylpyrrolidone. The solid phase extraction cartridge preferably comprises from about 0.025 g to about 1 g of the polymer.

The present invention enables the solid phase extraction of one or more solutes from an aqueous solution, without prior wetting of the sorbent with an organic solvent. The method is versatile with respect to solute identity, resulting in extraction of a broad range of solutes of varying polarity. A particular advantage of the method is that the sorbent can dry out during the extraction procedure without diminishing the ability of the sorbent polymer to retain solutes. Thus, the present invention provides a simpler method for the preparation of analytical samples, decreasing sample preparation

time and increasing sample throughput. The present method is, thus, also more amenable to automation than currently used methods.

BRIEF DESCRIPTION OF THE INVENTION

The Figure is a schematic, in cross-section, of one embodiment of the solid phase extraction cartridge of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for solid phase extraction of aqueous or buffered aqueous solutions which does not require that the sorbent be wetted with an excess of organic solvent prior to and during the solid phase extraction process. The invention is based on the discovery that polymers or resins comprising both a hydrophilic monomer and a hydrophobic monomer in a suitable ratio can be wetted by water while maintaining surprisingly effective retention of organic solutes with a wide range of chromatographic polarities.

As described in the Exemplification, a relatively small increase in the N-vinylpyrrolidone content of a poly (divinylbenzene-co-N-vinylpyrrolidone) copolymer resulted in a dramatic improvement in retention of polar organic solutes under conditions in which the pre-wetted polymer was dried under reduced pressure for several minutes. For example, under these conditions, recovery of acetaminophen from such a copolymer comprising 9 mole percent N-vinylpyrrolidone was 10.4%. Increasing the mole percent N-vinylpyrrolidone in the copolymer to 13 resulted in a 92%. recovery of acetaminophen. Similar results were observed for procainamide, ranitidine, and caffeine. For relatively nonpolar solutes the difference in recovery between the two copolymers was less dramatic.

The ability of poly(divinylbenzene-co-N-vinylpyrrolidone) copolymers comprising between 13 mole percent and 22 mole percent N-vinylpyrrolidone to retain organic solutes was also compared with that of octadecyl (C_8)-bonded silica gel. As discussed in the Exemplification, the C_8-bonded silica sorbent showed poor retention of polar organic solutes when the sorbent was pre-wetted with an organic solvent and then dried under reduced pressure prior to extraction. For example, this sorbent showed a 2.8%. recovery of m-toluamide under these conditions. In contrast, the poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer comprising 13 mole percent N-vinylpyrrolidone displayed a 96.3% recovery of m-toluamide under similar conditions. Overall, the results demonstrate that a balance between the mutually exclusive properties of water-wettability and retention of organic solutes can be achieved in a copolymer which has a suitable ratio of hydrophilic monomers and hydrophobic monomers.

In one embodiment, the invention is a method for removing a solute from a solution. The method comprises the step of contacting the solution with a water-wettable polymer formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer, whereby the solute is adsorbed onto the polymer. The solution can comprise water, or a mixture of water and a water-miscible polar organic solvent such as methanol, ethanol, N, N-dimethylformamide, dimethylsulfoxide or acetonitrile. The solution can also comprise a mixture of water or an aqueous buffer and a polar, water-miscible organic solvent. In a particularly preferred embodiment, the solution is an acidic, basic or neutral aqueous or predominately aqueous, i.e., greater than about 50% water by volume, solution. The solute is preferably an organic compound.

The solution can be contacted with the polymer in any fashion which permits intimate contact of the polymer and the solution, such as a batch or chromatographic process. For example, the solution can be forced through a porous polymer column, disk or plug, or the solution can be stirred with the polymer, such as in a batch-stirred reactor. The solution can also be added to a polymer-containing well of a microtiter plate. The polymer can take the form of, for example, beads or pellets. The solution is contacted with the polymer for a time period sufficient for the solute of interest to substantially adsorb onto the polymer. This is typically the time necessary for the solute to equilibrate between the polymer surface and the solution. The adsorption or partition of the solute onto the polymer can be partial or complete.

A preferred polymer for use in the present method is water-wettable and has the ability to retain a variety of solutes of varying polarity. The term "water-wettable", as used herein, describes a material which is solvated, partially or completely, by water. The material, thus, engages in energetically favorable or attractive interactions with water molecules. These interactions increase the amount of surface area of the material which, upon contact with water, is accessible to water molecules, and, hence, to solutes present in aqueous solution.

The term "monomer", as used herein, refers to both a molecule comprising one or more polymerizable functional groups prior to polymerization, and a repeating unit of a polymer. A polymer can comprise two or more different monomers, in which case it can also be referred to as a copolymer. The "mole percent" of a given monomer which a copolymer comprises is the mole fraction, expressed as a percent, of the monomer of interest relative to the total moles of the various (two or more) monomers which compose the copolymer.

In one embodiment of the method, the solution is contacted with the polymer in dry form. In another embodiment the polymer is wetted prior to contacting the solution with the polymer, for example, by treating the polymer with a polar organic solvent, followed by water or an aqueous buffer.

The hydrophilic monomer can comprise hydrophilic group. In one embodiment, the hydrophilic group is a heterocyclic group, for example, a saturated, unsaturated or aromatic heterocyclic group. Suitable examples include nitrogen-containing heterocyclic groups such as pyrrolidonyl and pyridyl groups. In another embodiment, the hydrophilic moiety is an ether group. The hydrophilic monomer can be, for example, N-vinylpyrrolidone, 2-vinylpyridine, 3-vinylpyridine, a hydrophobic moiety, 4-vinylpyridine or ethylene oxide.

The hydrophobic monomer can comprise, for example, an aromatic carbocyclic group, such as a phenyl or phenylene group, or an alkyl group, such as a straight chain or branched C_2-C_{18}-alkyl group. Suitable hydrophobic monomers include, but are not limited to, styrene and divinylbenzene.

In a preferred embodiment, the polymer to be contacted with the solution is a poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer. The polymer can comprise about 12 mole percent or more N-vinylpyrrolidone. In a particularly preferred embodiment, the polymer comprises from about 15 mole percent to about 30 mole percent N-vinylpyrrolidone.

The polymer can be in the form of, for example, beads having a diameter in the range from about 5 to about 500 μm,

preferably from about 20 to about 200 μm. The copolymer, preferably, has a specific surface area in the range from about 200 to about 800 square meters per gram and pores having a diameter ranging from about 0.5 nm to about 100 nm.

The solution comprising the solute can, optionally, further contain one or more additional solutes. In one embodiment, the solution is an aqueous solution which includes a complex variety of solutes. Solutions of this type include blood plasma, urine, cerebrospinal fluid, synovial fluid and other biological fluids, including extracts of tissues, such as liver tissue, muscle tissue, brain tissue and heart tissue. Such extracts can be aqueous extracts or organic extracts which have been dried and subsequently reconstituted in water or in a water/organic mixture.

The solution can also be ground water, surface water, drinking water or an aqueous or organic extract of an environmental sample, such as a soil sample. The solution can further be a food substance, such as a fruit or vegetable juice or milk or an aqueous or aqueous/organic extract of a food substance, such as a fruit, vegetable, cereal, or meat.

The solute can be any organic compound of polarity suitable for adsorption onto the polymer. Such solutes can include, for example, drugs, pesticides, herbicides, toxins and environmental pollutants resulting from the combustion of fossil fuels or other industrial activity, such as metal-organic compounds comprising a heavy metal such as mercury, lead or cadmium. The solutes can also be metabolites or degradation products of the foregoing materials. The solutes can also include biomolecules, such as proteins, peptides, hormones, polynucleotides, vitamins, cofactors, metabolites, lipids and carbohydrates.

In one embodiment of the method, the polymer is packed as particles within an open-ended container to form a solid phase extraction cartridge. The container can be, for example, a cylindrical container or column which is open at both ends so that the solution can enter the container through one end, contact the polymer within the container, and exit the container through the other end. The polymer can be packed within the container as small particles, such as beads having a diameter between about 5 μm and about 500 μm, preferably between about 20 μm and about 200 μm. The polymer particles can also be packed in the container enmeshed in a porous membrane.

The container can be formed of any material which is compatible, within the time frame of the extraction process, with the solutions and solvents to be used in the procedure. Such materials include glass and various plastics, such as high density polyethylene and polypropylene. In one embodiment, the container is cylindrical through most of its length and has a narrow tip at one end. One example of such a container is a syringe barrel.

The solid phase extraction cartridge can further comprise a porous retaining means, such as a filter element, or frit, at one or both ends of the cartridge adjacent to the polymer to retain the polymer within the cartridge and to remove undissolved solid materials from the solution as it flows into the cartridge, while still permitting solution flow into and out of the cartridge. Such a filter can be formed from, for example, fritted glass or a porous polymer, such as a porous high density polyethylene.

The amount of polymer within the container is limited by the container volume and can range from about 0.001 g to about 50 g, but is preferably between about 0.025 g and about 1 g. The amount of polymer suitable for a given extraction depends upon the amount of solute to be adsorbed, the available surface area of the polymer and the strength of the interaction between the solute and the polymer. This can be readily determined by one of ordinary skill in the art.

The present invention includes a solid phase extraction cartridge as described above, wherein the polymer is a water-wettable polymer formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer. The polymer can be, for example, a poly (divinylbenzene-co-N-vinylpyrrolidone) copolymer comprising about 12 mole percent or more N-vinylpyrrolidone. In a preferred embodiment, the copolymer comprises from about 15 mole percent to about 30 mole percent N-vinylpyrrolidone. The cartridge can be a single use cartridge, which is used for the treatment of a single sample and then discarded, or it can be used to treat multiple samples.

A preferred embodiment of the solid phase extraction cartridge of the present invention is illustrated in cross section in the Figure. Container **1** is a syringe barrel which can be formed of molded polypropylene and can have a volume ranging from about 1 cm^3 to about 50 cm^3. Water wettable polymer **2** is prepared by the copolymerization of N-vinylpyrrolidone and divinylbenzene and comprises from about 12 mole percent to about 30 mole percent N-vinylpyrrolidone. Polymer **2** is packed within the container as porous beads of diameter between about 20 μm and about 200 μm. The mass of polymer **2** packed within the container can range from about 0.025 g to about 10 g, depending upon the volume of the container. Frits **3** and **4** are formed of porous high density polyethylene.

The solution to be treated is added to the top of the solid phase extraction cartridge and allowed to flow through the cartridge, bringing the solute to be adsorbed into contact with the polymer. The solution can flow through the cartridge under the force of gravity. Increased flow rates can be achieved by establishing a pressure difference between the ends of the cartridge. Such a pressure difference can be established by attaching a vacuum source to the lower end of the cartridge or by applying positive pressure to the upper end of the cartridge, for example, by applying a pressurized gas, such as air or nitrogen, to the top of the cartridge, or by compressing the air within the cartridge above the polymer with a piston or plunger. The flow rate of the solution through the cartridge can be adjusted by regulating the pressure difference across the cartridge. Suitable solution flow rates, given in terms of the linear velocity of the solution, range up to about 14 mm/second, but are preferably in the range from about 0.7 to about 3.5 mm/second.

Another aspect of the present invention is a method for forming a solution of a solute which is suitable for quantitative analysis. In one embodiment, the solute is of a polarity suitable for adsorption onto the polymer. The method comprises contacting a first solution which includes the solute with a polymer formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer, whereby the solute is adsorbed onto the polymer. This is followed by washing the polymer with a suitable, stronger solvent or mixture of solvents, thereby desorbing or eluting the solute from the polymer and forming a second solution which contains the solute. This second solution is suitable for the quantitative analysis of the solute.

The solution contacted with the polymer can comprise the solute of interest in dilute form, for example, at a concentration too low for accurate quantitation. By adsorbing the solute onto the polymer and then desorbing the solute with

a substantially smaller volume of a less polar solvent, a solution which includes the solute of interest can be prepared having a substantially higher concentration of the solute of interest than that of the original solution. The method also results in solvent exchange, that is, the solute is removed from a first solvent and re-dissolved in a second solvent

The polymer need not be pretreated or wetted prior to contacting the solution with the polymer. In one embodiment, the polymer is treated with a water-miscible organic solvent, followed by water or aqueous buffer, prior to contacting the solution with the polymer. In another embodiment, the solution is contacted with dry polymer, that is, the polymer is not wetted prior to treatment of the solution.

The solution contacted with the polymer can comprise a polar solvent and is preferably predominately, i.e. greater than 50% by volume, an acidic, basic or neutral aqueous solution or aqueous buffer. The solution can also comprise a water-miscible polar organic solvent such as methanol, ethanol, acetonitrile, N,N-dimethylformamide, or dimethylsulfoxide, or a mixture of such a solvent and water.

The solution comprising the solute of interest can further comprise one or more additional solutes. In one embodiment, the additional solute or solutes are more polar than the solute of interest, and, thus, adsorb more weakly to the polymer than the solute of interest. Such an additional solute can be desorbed from the polymer by washing the polymer with a solvent which does not desorb the compound of interest, thereby forming a solution of the additional solute or solutes which is substantially free of the solute of interest. A suitable solvent for the desorption of the additional solute will typically be sufficiently polar that it does not desorb the compound of interest.

After desorption of the additional solute or solutes, the compound of interest can be desorbed by washing the polymer with a suitable, i.e., less polar, solvent. This forms a solution of the organic solute which is substantially free from more polar solutes and is suitable for the quantitative analysis of the organic solute.

In one embodiment, the solute of interest adsorbs onto the polymer, but one or more additional solutes do not. Such an additional solute can be, for example, of sufficiently high polarity that it does not adsorb onto the polymer. The additional solute can also comprise large molecules, for example, macromolecules such as proteins, which are unable to pass through the pores within the polymer, and, thus, have access to only a small fraction of the overall polymer surface area. Such molecules are typically retained poorly, if at all, by the polymer.

In a further embodiment, the additional solute or solutes are less polar than the solute of interest and, thus, adsorb to the polymer more strongly than the compound of interest. The compound of interest can be weakly to moderately adsorbed or not adsorbed. If adsorbed, the solute of interest is desorbed from the polymer by washing the polymer with a solvent of sufficient polarity that it does not desorb the additional solute or solutes. Thus, the compound of interest can be desorbed from the polymer without desorbing the other solutes.

In one embodiment, the additional solute or solutes are also analytes of interest. Thus a series of solutes initially present in a solution can be separated, and solutions of each suitable for quantitative analysis can be formed using the method of the present invention. In this case, the solution is contacted with the polymer so that the solutes adsorb to the polymer. The solutes are then desorbed from the polymer in order of decreasing polarity (i.e., most polar solute first, followed by solutes of successively decreasing polarity) by washing the polymer with a sequence of solvents of decreasing polarity.

Polymers, solutions and solutes which are suitable for this method include those described above. Solvents which are suitable for desorbing the solute from the polymer will typically be polar water-miscible organic solvents, such as alcohols, for example, methanol, ethanol, and isopropanol, acetonitrile, acetone, and tetrahydrofuran, or mixtures of water and these solvents. The desorbing solvent can also be a nonpolar or moderately polar water-immiscible solvent such as dichloromethane, diethylether, chloroform, or ethylacetate. Mixtures of these solvents are also suitable. Preferred solvents or solvent mixtures must be determined for each individual case. A suitable solvent can be determined by one of ordinary skill in the art without undue experimentation, as is routinely done in chromatographic methods development (McDonald and Bouvier, supra, (1995); Snyder and Kirkland, *Introduction to Modern Liquid Chromatography*, New York: J. Wiley and Sons (1974)).

The methods of the present invention can be used to prepare solutions of a solute which are suitable for quantitative analysis via a variety of techniques, including high performance liquid chromatography, gas chromatography, gas chromatography/mass spectrometry, and immunoassay.

The sorbent polymers used in the methods of the present invention can be prepared via standard synthetic methods. For example, a poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer can be synthesized by copolymerization of divinylbenzene and N-vinylpyrrolidone using standard methods of free radical polymerization which are well known in the art. One method for forming copolymers of this type is disclosed in U.S. Pat. No. 4,382,124, issued to Meitzner et al., the contents of which are incorporated herein by reference. The composition of the resulting copolymer depends upon the starting stoichiometry of the two monomers and can be readily varied. The composition of the product copolymer in some cases will not be substantially the same as the proportion of the starting materials, due to differences in reactivity ratios among the monomers.

The invention will now be further and specifically described by the following example.

Exemplification

Materials

The model solutes procainamide, acetaminophen, m-toluidine, m-toluamide, propranolol, caffeine, and 2,7-dihydroxynaphthalene were obtained from Aldrich Chemical Company (Milwaukee, Wis.), while doxepin, ranitidine, and betamethasone-17-valerate were purchased from Sigma Chemical Company (St. Louis, Mo). The tC_8 bonded silica solid phase extraction cartridge was obtained from Waters Corporation (Milford, Mass., catalogue no. WAT054960). A poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer comprising about 9 mole percent N-vinylpyrrolidone was obtained from Waters Corporation (Porapak®R). Poly (divinylbenzene) was also obtained from Waters (Styragel®).

Preparation of poly(divinylbenzene-co-N-vinylpyrrolidone) copolymers

To a 3000 mL flask was added a solution of 5.0 g hydroxypropylmethylcellulose (Methocel E15, Dow Chemi-

cal Co., Midland, Mich.) in 1000 mL water. To this was added a solution of 175 g divinylbenzene (DVB HP-80, Dow), 102 g N-vinyl-2-pyrrolidone (International Specialty Products), and 1.85 g azobisisobutyronitrile (Vazo 64, Dupont Chemical Co, Wilmington, Del.) in 242 g toluene. The resulting biphasic mixture was stirred for 30 minutes at room temperature using sufficient agitation to form oil droplets of the desired micron size. The resulting suspension was then heated under moderate agitation to 70° C. and maintained at this temperature for 20 hours. The suspension was then cooled to room temperature, filtered and washed with methanol. The filter cake was then dried in vacuo for 16 hours at 80° C. The composition of the product polymer was determined by elemental analysis. Elemental analysis: N: 2.24%; mole percent N-vinylpyrrolidone: 20%.

A series of poly(divinylbenzene-co-N-vinylpyrrolidone) copolymers comprising about 13, 14, 16, and 22 mole percent N-vinylpyrrolidone was also prepared by this method by varying the starting ratio of the divinylbenzene and N-vinylpyrrolidone monomers.

A 50 mg amount of each polymer was packed into a 1 cc Sep-Pak Vac® cartridge container (Waters Corporation) having a polyethylene frit at both the inlet and the outlet of the polymer bed to form a solid phase extraction cartridge.

Method

Each model compound was dissolved in 20 mM phosphate buffer, pH 7, to form a solution having a concentration of 10 μg/mL.

Solid phase extraction of model solutes

The solutions of the model solutes were subjected to solid phase extraction on solid phase extraction cartridges conditioned under two sets of conditions. In both cases the cartridge was attached to a vacuum manifold and treated with 1 mL methanol. The vacuum was set to about 4" Hg, to give a methanol flow rate of 1 mL/minute. Under the first set of conditions ("wet conditions"), the vacuum was released when the methanol level reached the top of the sorbent. Under the second set of conditions ("dry conditions") the sorbent was allowed to dry out under vacuum following conditioning with methanol. As in the first method, the cartridge was treated with 1 mL methanol, at a flow rate, under reduced pressure (4" Hg), of about 1 mL/minute. When the methanol level reached the top of the sorbent, the vacuum was set to 10" Hg and maintained for 10 minutes to dry the polymer bed.

In both wet and dry cases, 1 mL of the model compound solution was applied to the cartridge at a flow rate of 1 mL/minute. A 1 mL portion of 20 mM phosphate buffer, pH 7 was then added at a flow rate of 1 mL/minute. A 1 mL portion of methanol was then added at a flow rate of 1mL/minute to desorb and eluate the model compound. To the eluate was added an internal standard, and the model compound within the eluent was quantitated by high performance liquid chromatography.

Results

The results are summarized in the table below, which lists polar compounds (procainamide, acetaminophen and ranitidine), moderately polar compounds (caffeine, m-toluamide, m-toluidine, 2,7-dihydroxynaphthalene, and propranolol) and nonpolar compounds (dipropylphthalate, doxepin and betamethasone-13-valerate). When the sorbent was poly(divinylbenzene) all compounds except doxepin showed greater than 89% recovery when the sorbent was conditioned under wet conditions. Recovery of doxepin, as shown, was significantly lower because this compound required greater than 1 mL methanol for quantitative elution. When the sorbent was treated under dry conditions, only dipropylphthalate and betamethasone valerate were recovered in greater than 80% yield, and recovery of procainamide, acetaminophen and ranitidine fell below 10%.

When the sorbent was tC_{18}-bonded silica, each compound tested was recovered in high yield (>85%) under wet conditions. Allowing the sorbent to dry out had negligible effect on the recovery of dipropylphthalate, doxepin and betamethasone valerate, but reduced the yield of caffeine, m-toluidine, m-toluamide, 2,7-dihydroxynaphthalene and propranolol to about 13% or less.

When the sorbent was a poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer, recovery of each compound was in the range of about 80–100% when the sorbent was kept wetted. When the copolymer composition was 9 mole percent N-vinylpyrrolidone, high recovery of the nonpolar compounds was noted under both wet and dry conditions. The more polar compounds were recovered in high yield under wet conditions but in sharply reduced yield under dry conditions. Recovery of these compounds under dry conditions dramatically increased when the N-vinylpyrrolidone component of the copolymer was increased to about 13 mole percent or greater. The recovery of these compounds under wet conditions was essentially invariant as the copolymer composition was changed.

TABLE

Comparison of SPE recoveries for various model compounds Analyses performed in triplicate. DVB = divinylbenzene, NVP = N-vinylpyrrolidone % NVP given as mole percent NVP.

	Percent Recovery (Average)					
	Poly(DVB)		Poly(DVB-co-NVP) 9% NVP		Poly(DVB-co-NVP) 13% NVP	
Compound	wet	dry	wet	dry	wet	dry
Procainamide	95.4	2.5*	90.8	4.9*		84.8*
Acetaminophen	98.0	2.0*	93.8*	10.4*		92.3
Ranitidine	95.0	5.4*	89.6	13.4*		99.7
Caffeine	95.6*	30.8*	101.0	25.6*	96.0	95.7
Toluamide	98.5	54.8*	101.1	74.4*	96.5	96.3
Toluidine	95.6	80.7*	102.8	96.8	97.6	98.0
2,7-Dihydroxynaphthalene	99.5	46.5*	103.6	89.6*	96.8	96.3
Propranolol	92.3	55.8*	102.1	94.5	94.2	92.4
Dipropylphthalate	91.5	99.2	192.1	101.6	93.1	100.0
Doxepin	47.5+	55.4+	85.3	86.8	77.5+	78.0
Betamethasone-13-valerate	89.1	83.6	93.3	97.1	85.9+	89.8

	Percent Recovery (Average)					
	Poly(DVB-co-NVP) 14% NVP		Poly(DVB-co-NVP) 16% NVP		Poly(DVB-co-NVP) 20% NVP	
Compound	wet	dry	wet	dry	wet	dry
Procainamide	94.2	84*	99.9	98.8	89.1	88.3
Acetaminophen	97.9	89.4*	104.5	104.4	96.2	94.8
Ranitidine	93.5	88.4	98.3	97.6	86.0	85.2
Caffeine	98.7	96.8			99.7	97.3
Toluatnide	100.8	96.4			100.0	97.0
Toluidine	96.5	93.4			94.0	93.2
2,7-Dihydroxynaphthalene	95.9	94.2			96.7	95.4
Propranolol					94.1	95.4
Dipropylphthalate					89.5	89.3
Doxepin					84.1	81.8
Betamethasone-13-valerate					92.7	87.0

TABLE-continued

Comparison of SPE recoveries for various model compounds Analyses performed in triplicate. DVB = divinylbenzene, NVP = N-vinylpyrrolidone % NVP given as mole percent NVP.

	Percent Recovery (Average)					
	Poly(DVB-co-NVP) 20% NVP		Poly(DVB-co-NVP) 22% NVP		tC_{18}	
Compound	wet	dry	wet	dry	wet	dry
Procainamide		92.3	85.7	93.3		
Acetaminophen		93.4	96.7	102.2		
Ranitidine		92.1	76.9	86.6		
Caffeine	97.7	98.2	99.2	95.4	103.9	1.5*
Toluamide	97.0	97.3	100.5	96.3	103.7	2.8*
Toluidine	95.1	96.7	91.9	90.3	101.7	13.4*
2,7-Dihydroxynaphthalene	95.7	94.5	94.4	90.0	102.8	0*
Propranolol	88.5	85.5	88.0	88.3	98.2	9.3*
Dipropylphthalate	88.9	86.0	89.1	98.5	92.2	98.4
Doxepin	84.7	77.5	78.6	79.9	95.1	104.0
Betamethasone-13-valerate	84.0	85.0	85.9	85.9	86.6	88.3

*Breakthrough in load/wash
+Requires greater than 1 mL methanol for complete elution

Equivalents

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed in the scope of the following claims.

What is claimed is:

1. A method for removing one or more solutes from a solution, comprising contacting the solution with a water-wettable polymer formed by copolymerizing at least one hydrophilic monomer and at least one hydrophobic monomer, said polymer comprising from about 12 mole percent to about 30 mole percent of the hydrophilic monomer, whereby the solute is adsorbed onto the polymer.

2. The method of claim **1** wherein the solution comprises a polar solvent.

3. The method of claim **2** wherein the polar solvent is water, an aqueous solution or a mixture of water and one or more polar organic solvents.

4. The method of claim **3** wherein the solution is blood plasma, urine, cerebrospinal fluid, synovial fluid, a tissue extract, groundwater, surface water, a soil extract, a food substance, or an extract of a food substance.

5. The method of claim **2** wherein the solvent is methanol, ethanol, acetonitrile, tetrahydrofuran, N,N-dimethylformamide or dimethylsulfoxide.

6. The method of claim **1** wherein the hydrophilic monomer comprises a heterocyclic group.

7. The method of claim **6** wherein the heterocyclic group is a pyrrolidonyl group or a pyridyl group.

8. The method of claim **7** wherein the hydrophilic monomer is selected from the group consisting of 2-vinylpyridine, 3-vinylpyridine, and 4-vinylpyridine.

9. The method of claim **7** wherein the hydrophilic monomer is N-vinylpyrrolidone.

10. The method of claim **1** wherein the hydrophobic monomer comprises a phenyl group, a phenylene group or a straight chain or branched C_2–C_{18}-alkyl group.

11. The method of claim **10** wherein the hydrophobic monomer is styrene or divinylbenzene.

12. The method of claim **1** wherein the polymer is a poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer.

13. The method of claim **12** wherein the polymer comprises from about 15 mole percent to about 30 mole percent N-vinylpyrrolidone.

14. The method of claim **1** wherein the polymer is within an open-ended container.

15. The method of claim **1** wherein the solute is a drug, pesticide, herbicide, biomolecule, toxin, pollutant or a metabolite or degradation product thereof.

16. The method of claim **15** wherein the biomolecule is a protein, vitamin, hormone, polypeptide, polynucleotide lipid or carbohydrate.

17. A method for forming a solution of a solute which is suitable for quantitative analysis, comprising the steps of:

(a) contacting a first solution which includes the solute with a water-wettable polymer formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer, said polymer comprising from about 12 mole percent to about 30 mole percent of the hydrophilic monomer, whereby the solute is adsorbed onto the polymer; and

(b) washing the polymer with a solvent or a mixture of solvents, thereby desorbing the solute from the polymer and forming a second solution of the solute, said second solution being suitable for quantitative analysis.

18. The method of claim **17** wherein the first solution comprises water, an aqueous solution or a mixture of water and one or more polar organic solvents.

19. The method of claim **17** wherein the solvent is a polar, water-miscible organic solvent or a mixture of water and a polar, water-miscible solvent.

20. The method of claim **19** wherein the solvent is selected from the group consisting of acetone, tetrahydrofuran, acetonitrile, methanol, ethanol, n-propanol and isopropanol.

21. The method of claim **17** wherein the solvent is a water-immiscible organic solvent.

22. The method of claim **21** wherein the solvent is selected from the group consisting of diethylether, ethylacetate, chloroform and dichloromethane.

23. The method of claim **17** wherein the hydrophilic monomer is N-vinylpyrrolidone.

24. The method of claim **17** wherein the hydrophobic monomer is divinylbenzene.

25. The method of claim **17** wherein the polymer is a poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer.

26. The method of claim **25** wherein the polymer comprises from about 15 mole percent to about 30 mole percent N-vinylpyrrolidone.

27. A method for forming a solution of a polar organic compound which is suitable for quantitative analysis, comprising the step of contacting an aqueous solution which includes the polar organic solute and one or more additional solutes, the additional solutes being less polar than the polar organic solute, with a water-wettable polymer formed by copolymerizing at least one hydrophobic monomer and at least one hydrophilic monomer, said polymer comprising from about 12 mole per cent to about 30 mole percent of the hydrophilic monomer, whereby the additional solutes are adsorbed onto the polymer and the polar organic solute remains in solution.

28. The method of claim **27** wherein the hydrophobic monomer is divinylbenzene.

29. The method of claim **27** wherein the hydrophilic monomer is N-vinylpyrrolidone.

30. The method of claim **27** wherein the polymer is a poly(divinylbenzene-co-N-vinylpyrrolidone) copolymer.

31. The method of claim **30** wherein the polymer comprises from about 15 mole percent to about 30 mole percent N-vinylpyrrolidone.

* * * * *

United States Patent [19]

Golias

[11] **4,341,635**

[45] **Jul. 27, 1982**

[54] **MICROCHROMATOGRAPHIC COLUMN AND METHOD**

[75] Inventor: **Tipton Golias, Beaumont, Tex.**

[73] Assignee: **Helena Laboratories Corporation, Beaumont, Tex.**

[21] Appl. No.: **216,888**

[22] Filed: **Dec. 16, 1980**

[51] **Int. Cl.**[3] **B01D 15/08**

[52] **U.S. Cl.** **210/656; 210/198.2**

[58] **Field of Search** 210/656, 659, 198.2

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Primary Examiner—John Adee

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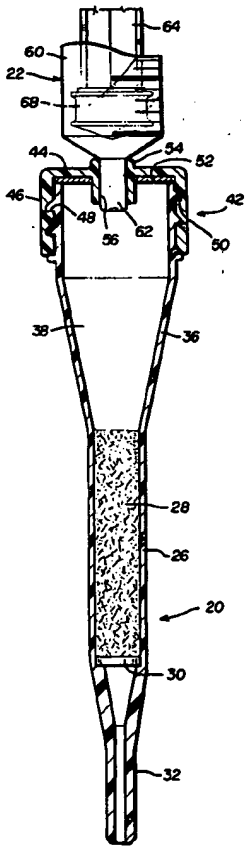
[57] **ABSTRACT**

A liquid microchromatographic column having a tubular reservoir portion receiving the fluid solution to be separated and discharging into a barrel portion containing finely divided ion exchange particles for separating the solution. A cap having an inlet port is received in sealed relation on the reservoir portion open end. The inlet port of the cap has a smooth generally cylindrical internal surface for receiving the discharge tip of a microsyringe or other pressure source, in sealed relation. The chromatographic method of this invention then includes introducing the fluid solution to be separated and an eluting solution into the reservoir. The discharge tip of a microsyringe is then inserted into the inlet port of the cap and the plunger of the syringe is depressed to increase the pressure in the reservoir and create a pressure drop across the ion exchange particles, substantially reducing the time required for chromatographic separation.

8 Claims, 2 Drawing Figures



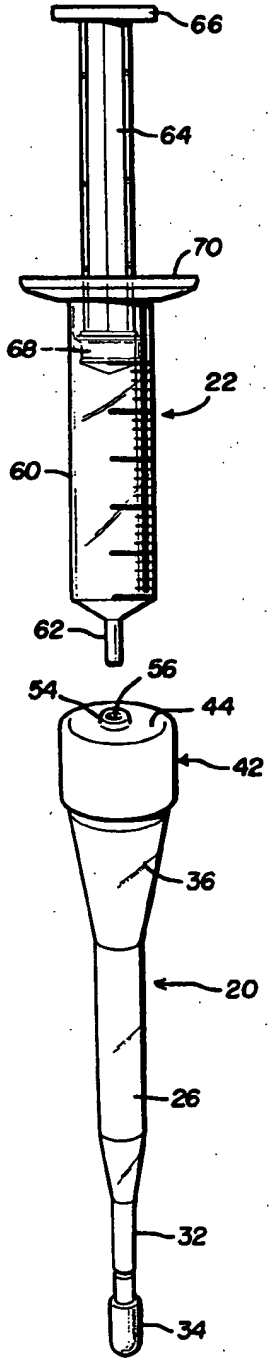


FIG. 1

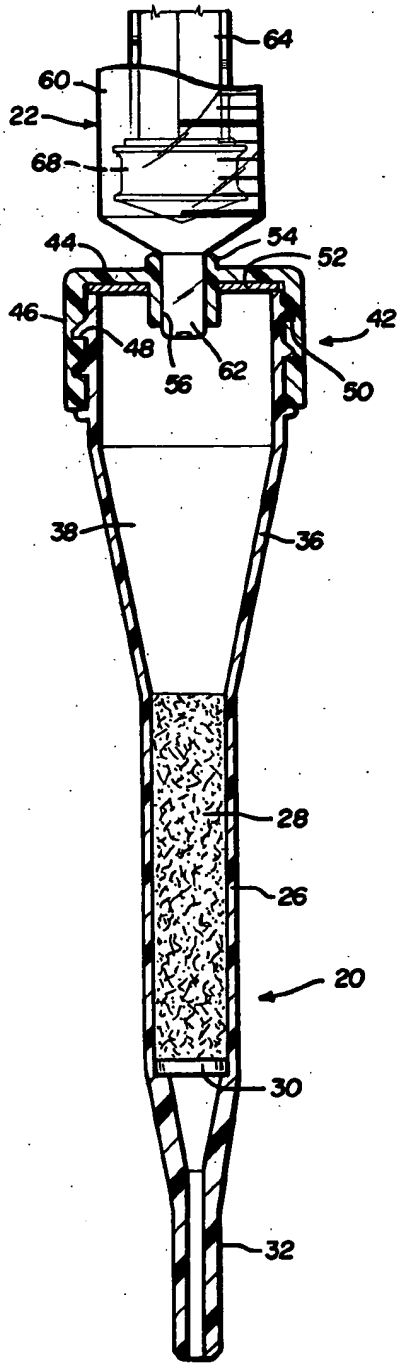


FIG. 2

MICROCHROMATOGRAPHIC COLUMN AND METHOD

FIELD OF THE INVENTION

Chromatography is a method of separating and analyzing small quantities of substances by passing a solution containing the substances through a column of finely divided particles which selectively adsorb the constituents of the solution. Initially, chromatography relied upon physical separation of the substances in one or more sharply defined colored bands. More recently, ion exchange chromatographic methods permit separation and quantitation of charged molecules, such as proteins and hemoglobins, making the procedure very useful in medical research and clinical evaluation.

Ion exchange resins are now commercially available in various pH ranges to selectively adsorb various molecules. The commercial ion exchange resins are a preparation of cellulose resin or other ion exchange resin particles which attract negatively charged molecules. Proteins, such as hemoglobins, contain many positive and negative charges due to the ionizing properties of the component amino acids. In anion exchange chromatography, the pH levels are controlled to cause different molecules to possess different net negative charges. The negatively charged molecules are attracted to the positively charged cellulose and bind accordingly.

Following binding, the molecules are selectively removed from the resin by altering the pH or ionic strength of the eluting solutions. The eluting solutions are used to selectively strip one component or molecule from the ion exchange resin column, leaving the remainder. This procedure can be used several times to separate a number of differently charged molecules.

Ion exchange columns and eluting solutions are also commercially available in kit form for separation and quantitation of various molecules, including hemoglobin A_{1c}, hemoglobin A_2, Vanillylamandelic Acid (VMA), Creatine Phosphokinase (CPK) MB, Lactic Dehydrogenase ($LDH_{1,2}$) and the molecular sieving and desalting chromatography used for Carcinoembryonic Antigen (CEA) assays. A more detailed description of the microchromatographic method of separation and quantitation of glycosylated hemoglobin is given hereinbelow as an example of the method and column of this invention, however it will be understood that the microchromatographic column and method of this invention may be used for any similar procedure.

A microchromatographic column generally includes a cylindrical barrel portion, which contains the ion exchange particles, and which discharges into a reduced diameter discharge tip portion, which is capped prior to use. The commercial microchromatographic columns generally include an enlarged reservoir portion, which serves as a funnel to receive the solution to be separated and which discharges into the barrel portion. The ion exchange particles are generally supported on a filter disc located between the barrel portion and the reduced diameter discharge tip portion. Microchromatographic columns of this type are available from the Assignee of the instant application, Helena Laboratories Corporation.

The glycosylated hemoglobin test is based upon the clinical determination that three minor components of norman human hemoglobin, HbA_{1a}, HbA_{1b} and HbA_{1c}, exhibit faster chromatographic mobilities than the main band of hemoglobin, HbA. Those minor components are collectively referred to as glycosylated hemoglobins, G-Hb, and differ from the major component HbA only by having a carbohydrate moiety attached to the N-terminal valine of the beta globin chain. It has now been discovered that the concentration of glycosylated hemoglobins, particularly HbA_{1c}, is related to the average blood sugar level in humans. A microchromatographic technique has been developed whereby the glycosylated hemoglobins may be quantitatively determined rapidly and simply in a liquid microchromatographic column. As described above, the negatively charged resin exhibits an affinity for positively charged molecules. At selective ion strength and pH, the glycosylated hemoglobins are less positively charged than hemoglobin A. Therefore, the glycosylated hemoglobins bind to the negatively charged resin less tightly than hemoglobin A. With the application of a first developing buffer, the glycosylated hemoglobins are eluted, while the other hemoglobin components are retained by the ion exchange resin. This fraction may then be compared to a total fraction or a second developing eluting buffer is used to elute the remaining hemoglobins to determine the percentage of glycosylated hemoglobins to total hemoglobins.

U.S. Pat. Nos. 4,142,855, 4,142,857 and 4,142,858, which are incorporated herein by reference, describe a chromatographic method of determining the concentration of glycosylated hemoglobins in blood and a microcolumn used for such tests. The microchromatographic column and method of this invention is an improvement upon the methods described in the referenced patents. However, as described hereinabove, the microchromatographic column and method of this invention are not limited to the determination of glycosylated hemoglobins.

The liquid chromatographic column and method described in the above referenced patents rely upon gravity for elution, requiring sixty to ninety minutes for the first elution and twenty to thirty minutes for the second elution. More recent improvements in microchromatographic columns by the assignee of this invention and others has limited the elution time to about twenty minutes, or about ten minutes for each elution. There is also an existing form of chromatography which utilizes high pressure to drive the eluting solution or developer. This method is called high pressure liquid chromatography or HPLC. The HPLC method however requires expensive equipment, costing several thousands of dollars, and operates under high pressures, which are not suitable for conventional microchromatographic columns. The need therefore remains for a simple, relatively inexpensive microchromatographic method which may be performed in seconds, rather than twenty to sixty minutes. The microchromatographic column and method of this invention achieves both purposes.

SUMMARY OF THE INVENTION

The liquid microchromatographic column of this invention includes a tubular barrel portion preferably terminating in a reduced diameter discharge tip as described above. The fluid solution to be separated is received in a tubular reservoir portion which discharges into the barrel portion and an open end. As described, the barrel portion contains the finely divided ion exchange particles for separating the fluid solution which is received in the reservoir portion. A cap is sealingly

received on the open end of the tubular reservoir portion. The cap includes an inlet port having a smooth generally cylindrical internal surface for receiving the cylindrical discharge tip of a conventional microsyringe or similar pressure source, in sealed relation. The microsyringe may thus be used to increase the pressure in the reservoir portion of the column and form a pressure drop across the ion exchange particles, substantially reducing the time required for chromatographic separation.

In the preferred embodiment, the cap is generally cup-shaped having an internally threaded lip portion and the cap is threadably received on the tubular open end of the reservoir portion. The cap preferably includes a sealing means, such as a sealing disc, to seal the reservoir upon receipt of the microsyringe. A microsyringe is normally plastic and the cylindrical internal surface of the inlet port is also preferably plastic to form a good seal between the discharge tip of the syringe and the reservoir of the microcolumn. A conventional microsyringe may be utilized in the method of this invention, including a barrel portion and a plunger. Depression of the syringe plunger, with the syringe discharge tip in the column inlet port increases the pressure in the reservoir to form a pressure drop across the ion exchange particles, as described above.

The method of this invention therefore includes introducing the fluid solution to be separated and an eluting solution into the column, through the open end of the reservoir. The discharge tip of a microsyringe is then inserted into the inlet port of the cap and the plunger of the syringe is depressed to create a pressure drop across the ion exchange particles, as described above. This method reduces the time for chromatographic separation from about ten to thirty minutes per elution to about ten seconds. Where two or more elutions are required, the time is reduced from about twenty to sixty minutes, to about twenty seconds. The time savings can be of tremendous advantage for certain assays. For example, the method of this invention will allow a doctor to evaluate data during the patient's office visit, rather than at a later time, which may be extremely important in immediate patient care.

Other advantages and meritorious features of the present invention will be more fully understood from the following detailed description of the microchromatographic column and method of this invention and the drawings, a brief description of which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a side elevation of the microchromatographic column of this invention with a conventional microsyringe; and

FIG. 2 is a cross-sectional side view of the microchromatographic column of FIG. 1 with a partial cross-sectional view of the microsyringe in the operating position.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS AND METHOD

As described above, the microchromatographic column 20 of this invention is adapted to separate fluid solutions under the pressure generated by a microsyringe 22, reducing the time of elution by a factor of about sixty. The disclosed microchromatographic column includes a cylindrical barrel portion 26 containing ion exchange resin particles 28. As described above, the particles are adapted to separate molecules in the column on the basis of charge. In the preferred embodiment, the column bed comprises a suspension of particles of a microgranular ion exchange cellulose. In the glycosylated hemoglobin test described above, the column comprises granular cation exchange resin equilibrated in phosphate buffer to pH 6.70 with 0.065% KCN. Further details of a suitable column are described in the above referenced U.S. patents, the disclosure of which is incorporated herein. In the disclosed microchromatographic column, the particles are supported on a suitable filter disc 30 located between the cylindrical discharge portion 26 and the reduced diameter discharge tip portion 32. A suitable filter disc is a high density polyethylene disc of the Ziegler type. A cap 34 is received on the end of the discharge tip to seal the tip prior to use. In the disclosed embodiment of the column, the fluid solution to be separated is received in the enlarged reservoir portion 36, which discharges into the barrel portion 26 and contains the supernatant 38, following filtration.

In the microchromatographic column of this invention, the reservoir is closed by an end cap 42 including an end portion 44 and a cylindrical lip portion 46. In the disclosed embodiment, the lip portion is internally threaded at 48 to be threadably received on the externally threaded end portion 50 of the reservoir. A sealing disc 52 is preferably received between the end of the tubular reservoir portion 36 and the end portion 46 of the cap. The cap may be formed of any suitable plastic including polyethylene or polystyrene. Any suitable sealing material may be utilized, including paper, cork and plastic materials. The end portion of the cap includes an inlet port 54 having a smooth generally cylindrical internal surface 56 to receive the discharge tip of a microsyringe or a similar pressure source, such as a rubber bulb, in sealed relation, as described hereinbelow. The internal surface of the inlet port may be slightly conical to form a tighter seal with the microsyringe. The disclosed microsyringe 22 is conventional and may be purchased commercially from a number of suppliers. The disclosed microsyringe includes a barrel portion 60 which is calibrated by volume. A typical microsyringe of this type has a volume of five cubic centimeters. The barrel portion terminates in a reduced diameter cylindrical discharge tip 62 having a smooth exterior surface. A plunger 64 is received in the barrel portion. A typical plunger is cruciform shaped having a flat end portion 66 and a head portion 68. In the disclosed embodiment, the head portion includes a soft polyurethane head having a plurality of radially extending cylindrical flange portions which sealingly engage the internal cylindrical surface of the barrel portion and force air or fluid from the barrel portion through the discharge tip 62. The disclosed embodiment of the microsyringe also includes a pair of opposed radially extending finger grip portions 70, which are integral with the barrel portion.

The chromatographic separation method of this invention comprises introducing the fluid solution to be separated and an eluting solution into the reservoir 36 of the microchromatographic column 20. The solution is then received in the cylindrical barrel portion 26 into the finely divided ion exchange particles or granules 28. As described above, the ion exchange resin is negatively charged and will covalently couple to small positively charged molecules. In the glycosylated hemoglobin test described above, the hemoglobins will adhere to the negatively charged ion exchange particles to be selec-

tively stripped by the eluting solutions. In the hemoglobin test, the pH or ionic strength of the first eluting solution is chosen to selectively remove the glycosylated hemoglobins, as described in the above referenced patents. The sample and the eluting solutions may be received through the open end of the reservoir, with the cap removed or through the inlet port **54** of the end cap, using a micropipette. In the test described in the above referenced patents, the solutions flow through the ion exchange cellulose particles **28**, solely under the influence of gravity. As stated therein, this procedure takes sixty to ninety minutes.

In the method of this invention, the time required for chromatographic separation is substantially reduced by using the pressure generated by the microsyringe **22**. As shown in FIG. **2**, the discharge tip **62** of the microsyringe is inserted into the inlet port **54** of the end cap **42**. The smooth cylindrical surface **62** of the discharge tip is received within the smooth internal generally cylindrical surface **56** of the inlet port in sealed relation and the plunger **64** is depressed, forcing air through the discharge tip of the syringe and increasing the pressure in the reservoir **36**. This creates a pressure drop across the ion exchange cellulose particles **28** and reduces the time required for chromatographic separation to about ten seconds. Where a second eluting solution is utilized, the procedure is repeated, including introducing the second eluting solution into the reservoir **36** and inserting the discharge tip **62** of the microsyringe into the inlet port **54** of the end cap and depressing the plunger **64** of the microsyringe to create a pressure drop across the ion exchange cellulose particles **28**.

The following Examples illustrate the time savings using the microsyringe assisted chromatographic test of this invention, wherein a pressure column created by the microsyringe is compared to a conventional gravity column of the improved type referred to hereinabove. It will be noted that the total elution time in each of the Examples is twenty seconds, compared to a total elution time for the gravity column of twenty minutes. For example, the time savings of a microsyringe assisted chromatographic test for hemoglobin A_{1c} will allow a doctor to evaluate the data during the patient's office visit, rather than at a later time. This could be very important in adjusting the patient's blood sugar level.

EXAMPLE 1

Established Range: 6.0–8.0
Mean Value: 7.0

Data Section

GLYCOSYLATED VALUES	
Pressure (Microsyringe) Column	Control (Gravity) Column
Col. #1–6.5	Col. #1–7.2
Col. #2–7.0	Col. #2–7.3
Col. #3–6.8	Col. #3–6.7
Col. #4–6.9	
Col. #5–6.7	
Mean - 6.8	Mean - 7.0
Standard Deviation - .19	Standard Deviation - .32
Coefficient of Variation - 2.8%	Coefficient of Variation - 4.5%
Elution Time: 2 times	Elution Time: 2 times
10 seconds = 20 seconds	10 minutes = 20 minutes

EXAMPLE 2

Established Range: 5.2–7.2
Mean Value: 6.2

Data Section

GLYCOSYLATED VALUES	
Pressure (Microsyringe) Column	Control (Gravity) Column
Col. #1–6.3	Col. #1–6.3
Col. #2–5.8	Col. #2–6.5
Col. #3–6.3	Col. #3–6.5
Col. #4–6.1	
Col. #5–5.9	
Col. #6–6.1	
Mean - 6.1	Mean - 6.4
Standard Deviation - .20	Standard Deviation - .12
Coefficient of Variation - 3.4%	Coefficient of Variation - 1.8%
Elution Time: 2 times	Elution Time: 2 times
10 seconds = 20 seconds	10 minutes = 20 minutes

EXAMPLE 3

Data Section

GLYCOSYLATED VALUES	
Pressure (Microsyringe) Column	Control (Gravity) Column
Col. #1–8.4	Col. #1–7.9
Col. #2–8.0	Col. #2–8.0
Col. #3–7.9	Col. #3–8.4
Col. #4–8.1	
Col. #5–8.1	
Col. #6–7.5	
Col. #7–7.7	
Col. #8–7.5	
Mean - 7.9	Mean - 8.1
Standard Deviation - .32	Standard Deviation - .26
Coefficient of Varation - 4.0%	Coefficient of Varation - 3.3
Elution Time: 2 times	Elution Time: 2 times
10 seconds = 20 seconds	10 minutes = 20 minutes

The "glycosylated values" listed for each chromatographic column tested in each of the above Examples are the percentages of glycosylated hemoglobin of the total hemoglobins. The method is briefly described hereinabove and described in more detail in the publications of the assignee and the above referenced U.S. patents. The determination is made using a standard laboratory spectrophotometer. The following formula was used to calculate the percentage of glycosylated hemoglobin for each column, using the method described above:

$$\frac{\text{Optical Density of the Fast Fraction Column}}{5\ (\text{Optical Density of Total Fraction Column})} \times 100 = \text{percentage glycosylated hemoglobin}$$

The optical density or absorbance of the contents of the fast fraction column, which is eluted with the first eluting solution and the total fraction collected with the second eluting solution was measured at a wave length of 415 nanometers. The dilution factor of 5 in the formulation is based upon the difference in volume between the total fraction of 15 milliliters and 3 milliliters of the fast fraction. More information regarding the method of calculation may be obtained from the published literature of the Assignee regarding the present gravity microchromatographic method used for quantitation of glycosylated hemoglobin.

As described above and established in the above Examples, the method of this invention substantially reduces the time required for chromatographic separation and quantitation of various solutions utilizing an ionic

microchromatographic column and method. The Examples establish that the method of this invention is as accurate as the conventional gravity separation, however the time is reduced from twenty minutes to twenty seconds. This is a very important improvement in chromatographic separation, particularly for patient clinical evaluations, where time may be of the essence. It will be understood that various modifications may be made to the microchromatographic column of this invention which has been adapted for use with a conventional microsyringe, which substantially reduces the cost of the apparatus and method of this invention.

I claim:

1. A liquid microchromatographic column having a tubular barrel portion terminating in a reduced diameter discharge tip, a tubular reservoir portion discharging into said barrel portion having an open end for receiving the liquid solution to be separated, said barrel portion containing finely divided ion exchange particles for separating the fluid solution received in said reservoir portion, and a cap received on said reservoir portion open end in sealed relation, said cap having an end portion including an integral generally cylindrical portion extending through said cap end portion on opposite sides of said end portion having an inlet port extending therethrough discharging into said reservoir portion, said inlet port having an elongated conical smooth internal surface closely receiving the cylindrical discharge tip of a hand operated pressure means in sealed relation for increasing the pressure in said reservoir and forming a pressure drop across said ion exchange particles, thereby reducing the time required for chromatographic separation.

2. The liquid microchromatographic column defined in claim 1, characterized in that said cap is cup-shaped having an internally threaded lip portion threadably received on said open end of said tubular reservoir portion, said cap including a sealing means sealing the communication between said inlet port of said cap and said reservoir portion of said column.

3. The liquid microchromatographic column defined in claim 1, characterized in that said cap is cup-shaped having an open end received over said reservoir open end and said sealing means comprising a sealing disc located between said reservoir open end and said end portion of said cap.

4. A liquid microchromatographic column having a tubular barrel portion terminating in a reduced diameter discharge tip, a tubular reservoir portion discharging into said barrel portion having an open end for receiving the fluid solution to be separated, said barrel portion containing finely divided ion exchange particles supported on a filter disc, said particles separating the fluid solution received in said reservoir portion, and a cap received on said reservoir portion open end in sealed relation, said cap being cup-shaped having an end portion and an internally threaded lip portion threadably received on said reservoir portion open end, and said cap end portion having an integral generally cylindrical portion extending through said cap end portion on opposite sides of said end portion having an inlet port extending through said cylindrical portion communicating with said reservoir portion, said inlet port having an elongated smooth generally cylindrical internal surface receiving the cylindrical discharge tip of a microsyringe in sealed relation for increasing the pressure in said reservoir and forming a pressure drop across said ion exchange particles and forcing fluid through said ion exchange particles, thereby reducing the time required for chromatographic separation.

5. The liquid microchromatographic column defined in claim 4, characterized in that said cap includes a sealing disc located between said cap end portion and said open end of said tubular reservoir portion.

6. A method of separating fluid solutions in a chromatographic column, said column having an open end for receiving the fluid solution, finely divided ion exchange particles supported in said column for separating the fluid solution and a cap received on said column open end in sealed relation having an elongated smooth, generally cylindrical inlet port, said method comprising:

a. introducing the fluid solution to be separated and an elutant into said column, through said open end, and

b. inserting the discharge tip of a hand operated pressure means into said cap inlet port in sealed relation and creating a pressure drop across said ion exchange particles by compressing said hand operated pressure means to force said eluent through said ion exchange particles, thereby reducing the time required for chromatographic separation.

7. The method of claim 6, wherein said pressure means is a syringe having a plunger, including depressing said plunger to create said pressure drop.

8. A method of separating a fluid solution in a microchromatographic column, said column having a tubular reservoir having an open end for receiving the solution to be separated, said reservoir discharging into a barrel portion of said column having finely divided ion exchange particles supported therein, and a cap closing said reservoir end portion in sealed relation, said cap having an inlet port communicating with said reservoir, the chromatographic method of separation comprising:

a. introducing the fluid solution to be separated and a first elutant into said reservoir, through said open end,

b. inserting the discharge tip of a microsyringe having a plunger into said cap inlet port in sealed relation with said inlet port and depressing said plunger to increase the pressure in said reservoir and create a pressure drop across said ion exchange particles, and

c. introducing a second fluid elutant into said reservoir, through said open end and inserting the discharge tip of said microsyringe into said cap inlet port and depressing said syringe plunger to create a pressure drop across said ion exchange particles to force said elutant through said ion exchange particles.

* * * * *

US006190559B1

(12) **United States Patent**
Valaskovic

(10) **Patent No.: US 6,190,559 B1**
(45) **Date of Patent: Feb. 20, 2001**

(54) **EVAPORATIVE PACKING A CAPILLARY COLUMNS**

(76) Inventor: **Gary A. Valaskovic**, 694 Green St., Apt. 3, Cambridge, MA (US) 02139

(*) Notice: Under 35 U.S.C. 154(b), the term of this patent shall be extended for 0 days.

(21) Appl. No.: **09/365,610**

(22) Filed: **Aug. 2, 1999**

Related U.S. Application Data

(62) Division of application No. 09/087,202, filed on May 29, 1998, now Pat. No. 5,997,746.

(51) **Int. Cl.**[7] **B01D 15/08**

(52) **U.S. Cl.** **210/656**; 210/198.2; 210/51.01; 95/82; 95/88; 96/101

(58) **Field of Search** 210/635, 656, 210/658, 198.2, 510.1; 95/82, 88; 96/101; 141/12, 80

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(74) *Attorney, Agent, or Firm*—Norris McLaughlin & Marcus P.A.

(57) **ABSTRACT**

Method for loading a column with a packing material by inserting one end of a column to be packed into a slurry of a packing material in a volatile solvent, allowing said slurry to be drawn into said end of said column by capillary action, withdrawing said end from said slurry, and removing said volatile solvent from the slurry that has been drawn into said end of said column, through the same end of the column at which the slurry entered, and sinteriing.

13 Claims, 5 Drawing Sheets

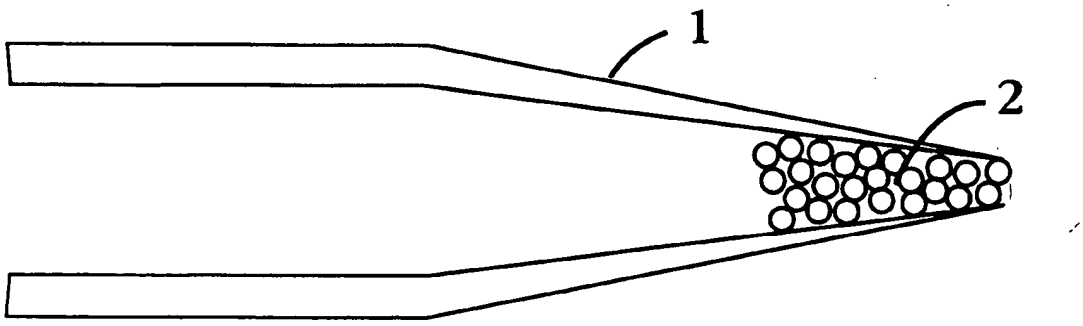


Fig. 1

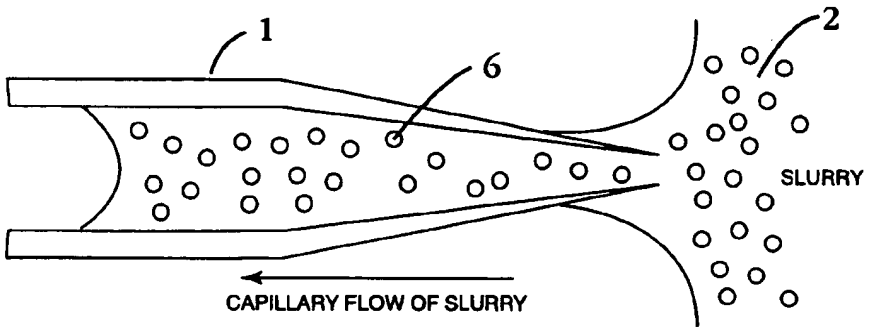


Fig. 2

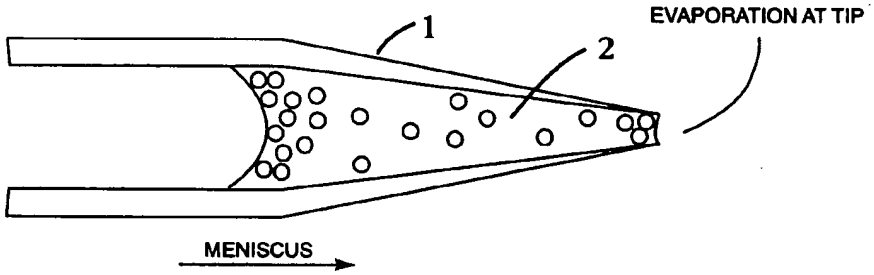


Fig. 3

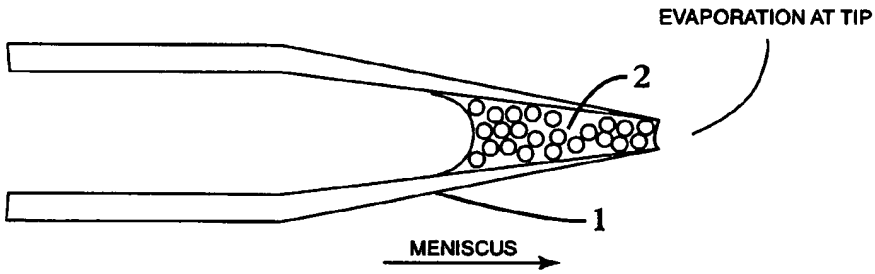
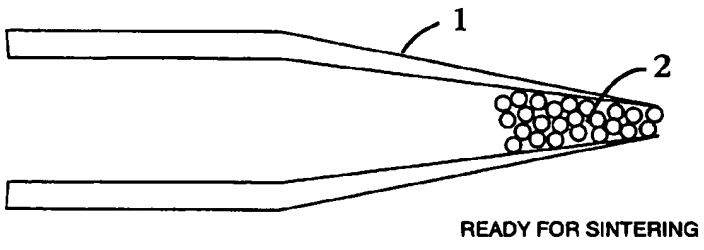


Fig. 4



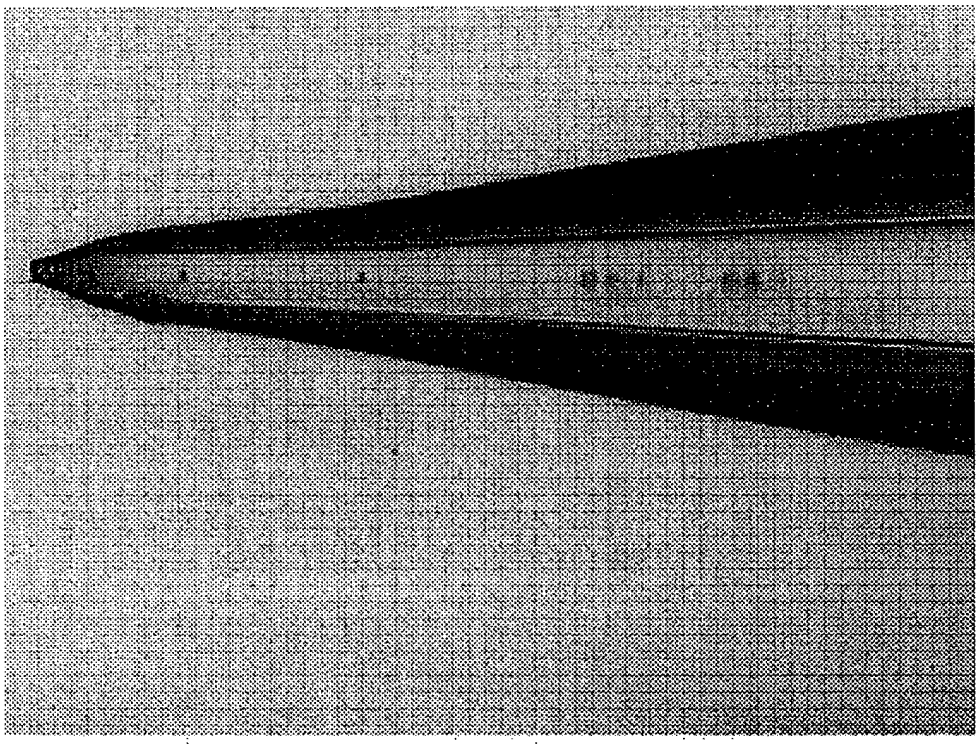


FIG. 5

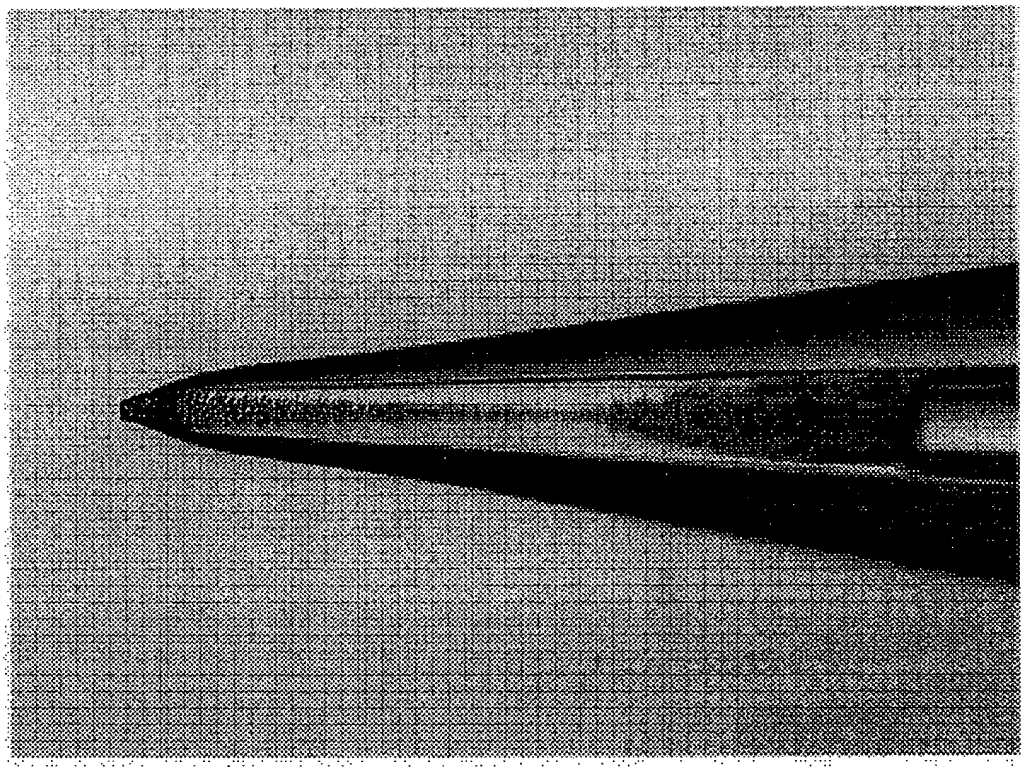


FIG. 6

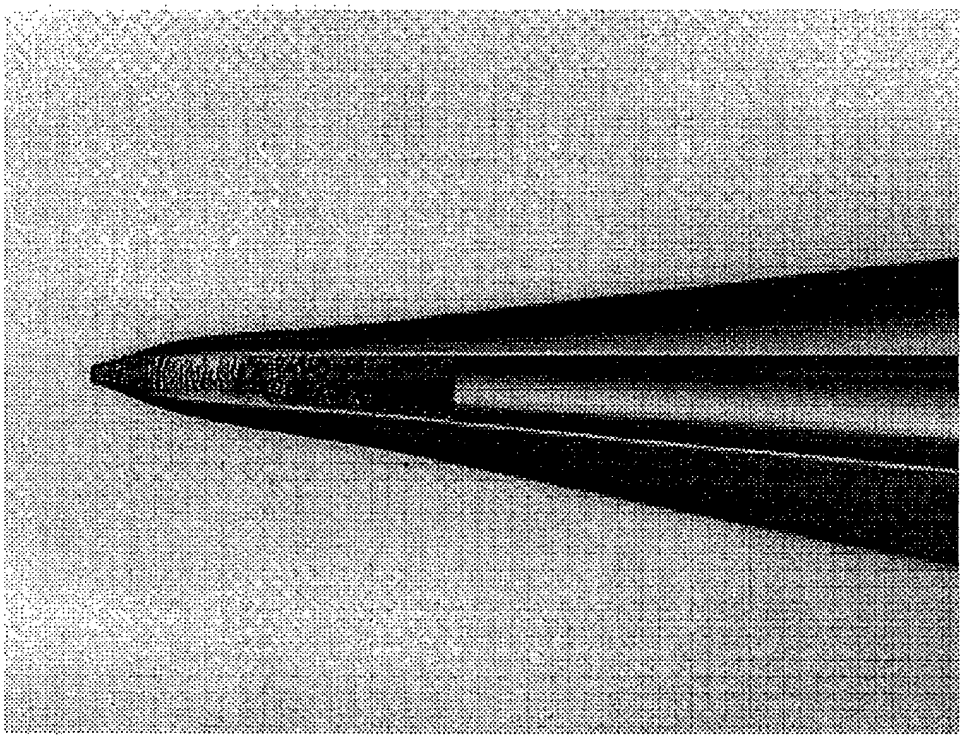
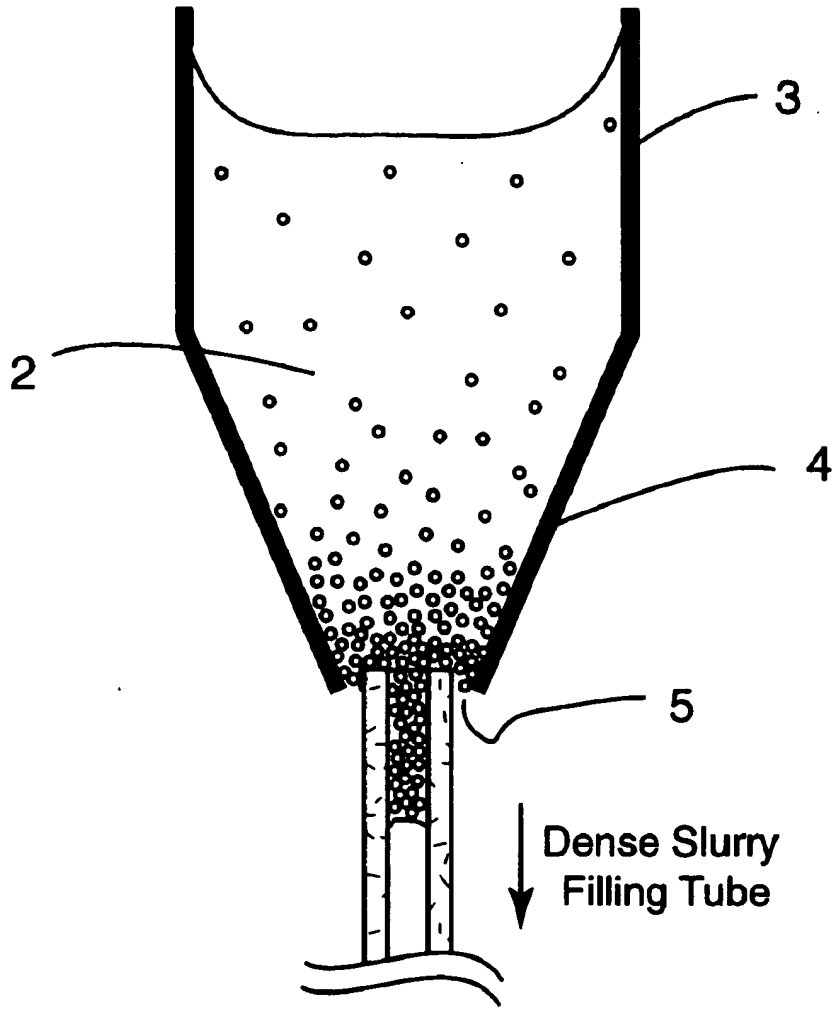


FIG. 7



Filling a tube with dense slurry by capillary action

Fig. 8

EVAPORATIVE PACKING A CAPILLARY COLUMNS

This application is a division of Ser. No. 09/087,202, filed May 29, 1998, now U.S. Pat. No. 5,997,746.

BACKGROUND OF THE INVENTION

This invention pertains to a novel method of packing capillary columns. More particularly, the invention pertains to a method of packing capillary columns by drawing a slurry of packing material into a capillary column through capillary action, and then removing solvent from the slurry through one end of the column.

There are a variety of methods currently in use for packing capillary columns, such as those columns used in the fields of chromatography and electrospray ionization mass spectrometry (ESI-MS). The most popular methods in current use are the so-called "slurry packing" methods.

U.S. Pat. No. 5,679,255 discloses a method whereby a retaining material, such as a ceramic frit which will allow solvent, but not packing material, to pass is placed in one end of the column. A slurry of polymeric packing material in an organic solvent, such as THF, is then pumped through the column, from the end opposite that having the retaining frit. The packing material thereby accumulates in the column, while the liquid portion of the slurry passes out through the frit. This method has certain disadvantages, however. The capillary tubing used must be capable of withstanding the pressure generated by the pumping of the slurry into the tube, and necessary equipment, such as a pump and solvent recovery system must be provided.

High packing pressure may also cause deterioration of or damage to the packing material.

U.S. Pat. No. 4,483,773 discloses a method wherein an end restriction is first placed in a column, to permit the flow of solvent, but restrict the passage of particles out of the end of the column. A slurry is then caused to flow into the column, under pressure. A two-step pressure sequence is then used to first fill up the column and form a bed of particles and then to uniformly compress the bed.

This method is less than completely satisfactory, because special equipment is required to practice it.

There is therefore a need for a simple, direct method for packing capillary columns, which does not rely on special equipment for pumping or pressurizing slurries into the columns.

SUMMARY OF THE INVENTION

It has now been discovered that a slurry of packing material can be drawn up into a capillary column through capillary action and that when the solvent in the slurry is evaporated out of the slurry through the same end of the column through which the slurry entered, the packing that is originally suspended in the slurry migrates towards the end of the column to become closely packed.

In accordance with the invention, there is therefore provided a method for packing a capillary column which comprises forming a slurry of the packing material to be packed into the column, placing the slurry in a vessel, inserting one end of a capillary column into said slurry in said vessel or reservoir, drawing slurry into said column by capillary action and removing the solvent from the slurry that has been drawn into the column through the same end through which it entered the column. Preferably, the solvent is removed only from the end through which it entered the column, and it is particularly preferable that the solvent be removed through only the same end through which it entered the column, by evaporation.

Finally, after the solvent has been removed from the slurry that has been drawn into the column, leaving only the packing material that was in the slurry, a portion of the packing at the end of the column is optionally sintered.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a cross sectional view of a capillary column **1** the end of which is inserted into a supply of slurry **2**, which has been drawn into the capillary column.

FIG. **2** is a cross sectional view of the capillary column of FIG. **1** after evaporation of a part of the solvent from slurry **2** through the same end of the column through which the slurry originally entered the column.

FIG. **3** is a cross sectional view of the capillary column of FIG. **2** after further evaporation has taken place.

FIG. **4** is a cross sectional view of the capillary column of FIG. **3**, after complete evaporation of the solvent has taken place.

FIG. **5** is a photograph of the end of a capillary column into which a slurry of packing material has been drawn by capillary action.

FIG. **6**. is a photograph of the capillary column of FIG. **5**, after partial evaporation of the solvent in the slurry has taken place, out of the end of the column.

FIG. **7** is a photograph of the capillary column of FIG. **6**, after further evaporation has taken place.

FIG. **8** is a cross sectional view of a vessel **3** having a conical bottom **4** with a hole **5** into which capillary **1** has been inserted whereby slurry **2** is drawn into column **1** by capillary action.

DETAILED DESCRIPTION

The slurry used in accordance with the invention is a slurry of a packing material in a volatile solvent. Such slurries are prepared by mixing a packing material with a solvent.

The packing materials may be particles of a variety of shapes, such as spherical, hemi-spherical, "irregular" spheres, rods with aspect ratios of <5:1, fractured "chips" (i.e., shapes associated with finely ground materials), precipitated crystallites (tiny cubes, prisms, dodecahedral, etc.). Spherical or nearly spherical shapes are preferred, however, since such shapes allow for the most uniform and dense packing. The packing materials may be solid, hollow or porous such as, for example, solid, hollow or porous spheres.

Preferred packing materials are ceramic, metallic or polymeric. The ceramic materials which can be used include, for example, soda-lime glass, borosilicate glass, porous silica (silica gel) and non-porous silica. The metals which can be used include, for example, colloidal gold, colloidal silver, nickel and stainless steel. The polymeric materials which can be used include, for example, fluoropolymers, such as polyvinylidene fluoride (PVDF), fluorinated ethylene propylene (FEP); styrenics, such as polystyrene (PS) and polystyrene/divinylbenzene copolymer (PS/DVB); polyolefins such as high density linear polyethylene (HDPE), low-density linear polyethylene (LDPE) and polypropylene; polyketones, such as polyetheretherketone (PEEK); acrylics, such as polymethylmethacrylate (PMMA) and vinyls, such as divinylbenzene (DVB). Particularly preferred materials

are borosilicate glass, silica (both porous silica and non-porous silica) and PS/DVB copolymer.

The particles which are used should have dimensions, i.e., diameters in the case of spheres, which are smaller than the smallest internal dimension of the column to be used, if the column has an internal shape other than round, or smaller than the internal diameter if the column, if the column to be used has a round internal shape, and should have maximum dimensions, or diameters if spherical, of about ½ the smallest internal dimension or diameter of the columns used. In general, the largest dimensions of non-spherical particles, or the diameters of the spherical particles used, range from about 0.1 μm to about 1 mm, although a range of 0.25 μm to about 250 μm is preferred; a range of 0.5 to 30 μm being particularly preferred, a range of 1 to 5 μm being especially preferred, and a range of 2 to 4 μm being very especially preferred.

There are many solvents known to the art which can be used to form the slurry. Preferred solvents are methanol, acetone and tetrahydrdrofuran (THF), although almost any volatile solvent can be used. The solvent selected should, of course, be one that will not dissolve, swell or otherwise harm the packing material selected, although it should "wet" the surface of the packing material.

The capillary tubes which are used for the columns are those known to the art, and can, for example, be those which are generally classified as ceramics, such as borosilicate glass, fused-silica, polyimide coated fused-silica and aluminum coated fused-silica; metallic, such as stainless steel, glass lined stainless steel or silica lined stainless steel; or they can be of polymeric materials. The polymeric material which can be used include fluoropolymers, such as ethylene tetrafluoroethylene (ETFE), fluorinated ethylene propylene (FEP) and polytetrafluoroethylene(PTFE); polyolefins, such as high density linear polyethylene (HDPE), low-density linear polyethylene (LDPE) and polypropylene; polyketones, such as polyetheretherketone (PEEK) and silica-lined PEEK; acrylics, such as polymethylmethacrylate (PMMA), polyamides, such as nylon 6, nylon 11 and nylon 12; and polyimide., Preferred capillary tubing for use as capillary columns in accordance with the invention are those of polyamide-coated fused silica, stainless steel, PEEK and HDPE, although polyimide-coated fused silica is especially preferred.

The internal or external shapes of capillary columns used in the practice of this invention can take on a variety of regular geometric shapes, such as round, oval, square, rectangular, polygonal, such as pentagonal, hexagonal, and the like; or can take on irregular shapes. The term "internal shape" of the capillary columns, as used herein, has the same meaning as the "bore" of a capillary column. Particularly preferred are those columns having a round internal shape or bore.

The columns used in the practice of the invention, having round internal shapes or bores, have inside diameters in the range of from about 1 μm to about 2 mm, preferably 5 to 250 μm and particularly preferably 20 to 100 μm. Where columns having internal shapes other than round are used, their internal cross-sectional areas should be in the same range as that of a column having a round internal shape with a diameter in the range of from about 1 μm to about 2 mm, preferably that of a column having a round internal shape with a diameter 5 to 250 μm and particularly preferably that of a column having a round internal shape with a diameter 20 to 100 μm

The columns can be of uniform internal dimensions or diameter over their entire length, such as those typically used as chromatography columns, or they can be tapered at one end, so that the internal diameter tapers to a narrow tip or needle, such as those columns used for electrospray ionization mass spectrometry (ESI-MS). The columns having tapered ends are also referred to in the art as needles. FIGS. 1–7 illustrate the ends, or tips, of the tapered-end columns, also referred to as needles. Where such tapered-end columns are used, the end or tip of the column, also referred to as the end of the needle, which is the end of the column where the packing is to be loaded, has an internal diameter ranging from about 1 μm to about 100 μm, preferably from 5 to 30 μm, particularly preferably 10 to 20 μm, especially preferred is a diameter of 15 μm. The length of the tapered portion, meaning the length of column over which the diameter tapers from the internal diameter of the untapered portion of the column to the internal diameter of the tip ranges from about 0.1 to 10 mm, preferably from 0.25 to 3 mm, especially preferably from 0.5 to 1.5 mm.

The length of the columns to be used will vary with the contemplated application, as well as the amount of additional packing, if any, which is to be used in combination with the packing of the present invention. That is to say, the packing of the present invention can be used alone, or in combination with other packings which can be added to the column before or after the present packing. Packed columns with lengths of 19 meters or more are known (U.S. Pat. No. 4,793,920), and such columns can be used in the practice of this invention, for which the length of the column used is not limited. As will be understood by those skilled in the art, however, the amount of packing that can be packed into a column using the method of this invention is limited by the capillarity between the slurry of packing material and the capillary column, which results in a specific theoretical maximum capillary height for each combination of slurry composition and capillary column composition, at given ambient conditions. The maximum capillary height for any particular application can easily be determined by simply inserting one end of the capillary column into the slurry, and observing how high into the column the slurry is drawn.

The slurry can be prepared by conventional methods, known to those skilled in the art. One such method is simple mixing, wherein a solvent is introduced into a vessel, such as a vial, beaker or a flask, together with the packing material, and the contents are then stirred. The capillary tube can then be inserted into the thus prepared slurry in the vial, beaker or flask, whereupon the slurry is drawn into the capillary tube by capillary action. The slurries according to the invention are formed with about 0.002 to 8 grams of packing per ml of solvent, preferably 0.03 to 5 grams of packing per ml of solvent, particularly preferably 0.2 to 1 gram packing per ml of solvent.

Alternatively, the slurry can be transferred from the vessel in which it was prepared into another vessel, such as a vessel having a conical bottom with a hole at the apex of the conical bottom, such as is shown in FIG. 8. One such vessel which can be used in accordance with the invention, is a common polyethylene pipette tip. In yet another embodiment of the invention, a vessel having a conical tip with a hole in the apex of the cone and a septum in the hole can be used. When using such vessels, the capillary tube is inserted into the slurry through the hole or, if the hole in the vessel is equipped with a septum, through the septum.

The time required to draw the slurry into the capillary tube by capillary action varies, depending upon a variety of factors, such as the dimensions of the packing material, i.e., the diameter of the spherical packing material, as well as the density of the slurry and the inside diameter of the capillary

tube. In general, however, the slurry will be drawn into the capillary virtually instantly upon insertion of the tube into the slurry. Depending upon the extent to which it is desired to fill the capillary tube with packing, more or less time will be required. If it is desired to pack only the tip of the capillary tube, to form a frit, for example, the desired amount of packing can be drawn in less than one second. If, on the other hand, it is desired to fill the column to the maximum achievable extent, known as the "full capillary height", several hours may be required. In general, however, the desired amount of slurry can be drawn into the capillary tube in from about 0.1 second to about 2 hours, although in most cases it will require only from about 0.1 second to about 30 minutes. The time required will, of course, vary depending upon the particular nature of the slurry and that of the capillary tube, as well as the conditions at which the filling is being conducted, such as temperature and pressure. Although it is possible to conduct the filling operation at elevated temperature and either elevated or reduced pressure, satisfactory results are generally obtained at ambient temperature and pressure.

Where no septum is used, the hole in the conical bottom of the aforedescribed vessel should be of such a size that the surface tension of the solvent in the slurry will prevent passage of slurry through the hole. Such holes will range in size from a diameter of about 0.1 mm to about 3 mm, preferably about 0.3 mm to about 1 mm.

A particular advantage of using such conical bottomed vessels is that the slurry, once placed in said vessel, can be allowed to settle, thereby forming a higher concentration of packing per ml of solvent in the bottom of the vessel, and the slurry that is drawn into the capillary tube from the vessel will thus have a higher concentration of packing than the original slurry.

A further advantage of using a conical bottomed vessel is that, as the slurry settles out in the conical bottom, a concentration gradient is formed, whereby the concentration of particles in the solvent gradually increases from the top of the vessel to the bottom. The density of the slurry that is drawn into a capillary tube inserted into the hole at the apex of the cone in the conical bottom of the vessel can then be controlled by varying how far the tube sticks up into the vessel. The closer the end of the capillary tube is to the bottom of the cone, the higher the density of the slurry drawn into the capillary tube will be.

In a particularly preferred embodiment, the slurry comprises glass microspheres as packing material in methanol as solvent, at concentration of about 0.2 grams of microspheres per ml of methanol.

In a particularly preferred embodiment of the invention, the packing material in the column is sintered, after the solvent has been removed. The packing is sintered by applying energy to it. This is done by heating the packing material with a heat source, such as hot air, by laser radiation, microwave heating or a combination of such heating means. The amount of heat applied is controlled to be sufficient to sinter the packing material, while avoiding melting it. A sufficient amount of heat must be applied so as to cause the packing material to soften. (For glass, silica or polymeric materials, the glass transition temperature must be reached.) Heating time and temperature must be sufficient to cause the particles to fuse together, but not so long or so hot as to eliminate all of the inter-particle spacing and voids. Complete melting of the packing material is, of course, to be avoided.

In the practice of this invention, the slurry is drawn into the capillary column, as described above, through capillary action. Then, the solvent that is in the slurry that has been drawn into the column is removed from the slurry through the same end of the column through which the slurry entered the column. Preferably, the solvent is removed from the slurry by evaporation. In conducting the evaporation of the solvent, the column can be allowed to stand in ambient air, as, for example may exist in a fume hood, or by passing a stream of a gas, such as air or nitrogen, over the tip of the end, to accelerate evaporation. The rate of evaporation can be controlled by such factors as the rate of gas which is passed over the tip of the end of the column, as well as the ambient temperature and the temperature of the gas that is passed over the tip of the end of the column.

Alternatively, the solvent can be removed through the end of the column by other means, such as "blotting", by holding an absorbent material, such as a filter paper or membrane, over the end of he column to contact and draw solvent out of the column.

In one embodiment of the method of the invention, a slurry that is close to the theoretical maximum density of particles suspended in a unit volume of solvent is used. In such a case, the dense slurry, when drawn into the end of the capillary tube, will remain tightly localized near the end of the capillary tube and, when the solvent is evaporated, the meniscus packs the slurry into a tight slug of material.

In yet another embodiment, the capillary tube is pre-filled with solvent, and then a portion of the solvent is permitted to evaporate, leaving an air gap at the end of the column. The end with the air gap is then inserted into the slurry, whereby the amount of slurry that is drawn into the column is limited by the air gap between the entering slurry and the pre-filled solvent. Then, the solvent, including both the pre-filled solvent and the solvent of the slurry is evaporated. As the solvent evaporates, the meniscus movement packs the packing material into a tight porous plug at the end of the tube.

Referring now to the drawings, FIG. **1** shows the end tip of a tapered-end capillary column **1**, also referred to as a needle, of the present invention. As shown, the capillary tube is inserted into a reservoir of slurry **2**, comprising packing spheres **6** dispersed in a solvent, and the slurry has been drawn into the tube by capillary action.

FIG. **2** shows the capillary tube end-tip of FIG. **1**, after partial evaporation of the solvent out of the end-tip. As is illustrated, the meniscus of the surface of the slurry inside the column and furthest removed from the end tip of the column has moved towards the end tip, as solvent is evaporated from the end tip. As also illustrated, and this is an important feature of the present invention, the meniscus, as it moves towards the end-tip, brings with it a concentration of the packing material, so that as the solvent is evaporated from the end-tip, the slurry concentrates itself towards the end-tip. This is entirely surprising, as the packing material would have been expected to "precipitate out" of the solvent, and be left behind in a dry state, as the volume of solvent shrinks towards the end tip. It was not to be expected that the moving meniscus would actually carry the packing material towards the end-tip, thereby concentrating the packing material in the direction of the end tip. It has now been discovered that as the liquid solvent evaporates, the meniscus traveling towards the end of the tube collects and gently forces the packing material towards the end of the tube. The surface tension of the liquid/air (or liquid/nitrogen) interface at the end of the tube is sufficient to hold the packing material in place in the slurry against this gentle moving force. The tube may optionally be vibrated and/or slowly rotated on its axis during the evaporation process to promote dense packing of the packing material.

FIG. 5 is an actual photograph of a capillary tube such as that illustrated in FIG. 2.

FIG. 3 illustrates the column of FIG. 2 after further evaporation has taken place, whereby the concentration of the packing material has been further increased and moved closer to the end-tip.

FIG. 6 is an actual photograph of a capillary tube such as that illustrated in FIG. 3.

FIG. 4 illustrates the column of FIG. 3 after all of the solvent has been evaporated. The packing material illustrated in FIG. 4, with the solvent removed, is ready for sintering.

FIG. 7 is an actual photograph of a capillary tube such as that illustrated in FIG. 4.

The method of the present invention can be better understood by reference to the following example, but is not limited thereby.

EXAMPLE 1

A 10 cm long piece of 360 μm outside diameter (OD), 75 μm inside diameter (ID) polyimide coated fused silica tubing (Polymicro Technologies, Phoenix, Ariz.) was mounted in a commercial laser-heated micropipette puller (Sutter Instruments, Novato, Calif.) and drawn down into two sharp needles so that the internal diameter at the needle end was reduced to 15 μm.

A slurry of 5 μm diameter solid glass microspheres was prepared by mixing 0.1 gram of the spheres with 0.5 milliliters of 100% methanol in a small (1 ml) glass vial. The mixture was stirred thoroughly for 5 minutes, ultrasonicated for 5 minutes, and then allowed to settle for 2 hours. After removing the excess solvent above the slurry, which had settled to the bottom of the vial, approximately 10 μl of slurry was transferred to a polyethylene vessel (a commercial polyethylene pipette tip) possessing a conical bottom having a 0.5 mm hole at it's apex. The slurry was again allowed to settle for approximately 5 minutes, while holding the pipette tip in the vertical position.

Both the slurry-containing vessel and the silica needle were mounted horizontally on the stage of a standard light microscope and viewed at 100-x magnification. The microscope stage had an additional translation stage that allowed the silica needle to be moved into the hole of the pipette tip. By moving the tip into brief contact (~0.5 seconds), with the slurry through the hole, approximately 75–100 microspheres were transferred into the silica needle.

The needle was allowed to rest on the stage of the microscope for 5 minutes while the methanol completely evaporated from the tip of the needle. During this time, the silica was packed into place by the movement of the meniscus.

The needle was transferred to a device holding a platinum foil heating element with a 3 mm "trough" filament, 3 mm wide (Sutter Instruments filament number FT330B). The end of the needle containing the packed spheres was centered in the filament. The filament was energized for 12 seconds with a heat output of 20.1 watts, to sinter the microspheres.

EXAMPLE 2

The procedure of Example 1 was repeated, except that a needle fabricated from 50 μm ID fused silica tubing pulled down to an 8 μm ID tip was used. Approximately 25–50 spheres were transferred into the end of the silica needle. For sintering, the heat output of the filament device was reduced to 19.4 watts.

EXAMPLE 3

The procedure of Example 1 was repeated, but a 10 cm piece of 50 μm ID, 360 μm OD polyimide coated, fused silica tubing was cut with a clean square end-face. The slurry was prepared as in Example 1, but 10 μm highly porous spheres of poly(styrene-divinylbenzene(Poros®, Perspective Biosystems 10-R2) were substituted for the glass microspheres. The column was loaded as described in Example 1. The packing was sintered in the same apparatus to form a frit, but the heating conditions were 3 seconds at 6.3 watts

EXAMPLE 4

A 25-cm length of 75 μm ID tubing was cleaved to have a flat end-face.

A slurry was prepared as in Example 1. After settling, 100 μl of slurry was transferred to a polyethylene pipette tip. The tip was held in the vertical position and the slurry allowed to settle for 15 minutes. Settling time was monitored with a light microscope to ensure that >95% of the material had settled out to the bottom of the tip.

While left in the vertical position, the silica capillary tube was inserted approximately 50 μm into the hole at the bottom of the pipette tip. The dense (nearly opaque) slurry filled the silica tube by capillary action. The tube was left in contact with the slurry until 15 cm of the tube was filled.

Upon removal, the end of the silica tube was brought into contact with a Nylon filter pad (Milipore Corp.), and mounted horizontally on the stage of a light microscope. The movement of the meniscus and packing of the column was monitored for approximately 45 minutes. When evaporation appeared to be complete, the Nylon filter paper was removed and packing material at the end of the tube was sintered into a frit by heating in the heating device described in Example 1, with a filament output of 25 watts for 12 seconds.

EXAMPLE 5

The procedure of Example 4 was repeated, except that the filling was accomplished with the tube in the horizontal position. A column with 10 cm of packed material was thereby fabricated.

The invention and its advantages are readily understood from the foregoing description. It is apparent that various changes can be made in the process without departing from the spirit and scope of the invention. The process as herein presented, is merely illustrative of preferred embodiments of the invention, and not a limitation thereof.

I claim:

1. A method for loading a column with a packing material which comprises forming a slurry of a packing material in a volatile solvent, inserting one end of a column to be packed into said slurry, allowing said slurry to be drawn into said end of said column by capillary action, withdrawing said end from said slurry, and removing said volatile solvent from the slurry that has been drawn into said end of said column, through the same end of the column at which the slurry entered.

2. The method of claim 1, wherein said solvent is removed from said slurry that has been drawn into said end of said column, by evaporation.

3. The method of claim 1, wherein said solvent is removed from said slurry that has been drawn into said end of said column, by absorption onto an absorbent.

4. The method of claim 1, wherein said absorbent is filter paper or membrane.

5. The method of claim 1, wherein said packing material is a sinterable packing material and wherein, after said

solvent has been removed, energy is applied to said sinterable packing to sinter it.

6. The method of claim 5, wherein said energy is in the form of heat, a laser beam or microwave radiation.

7. The method of claim 5, wherein said sinterable material is glass, silica, metal or polymer.

8. The method of claim 7, wherein said sinterable material is glass, in the form of spheres having a diameter of from about 0.25 μm to about 250 μm.

9. The method of claim 7, wherein said sinterable material is silica, in the form of spheres having a diameter of from about 0.25 μm to about 250 μm.

10. The method of claim 1, wherein said column is a hollow capillary tube of fused silica, having an inside diameter of from about 1 μm to about 2 mm.

11. The method of claim 10, wherein said inside diameter is from about 5 μm to about 250 μm.

12. The method of claim 1, wherein said end of said capillary tube is tapered.

13. The method of claim 12, wherein said capillary tube has an internal diameter of from about 20 μm to about 100 μm, and said tapered end is tapered to a tip having an internal diameter of from about 2 μm to about 30 μm.

* * * * *

US005589063A

United States Patent [19]

Sanford et al.

[11] Patent Number: 5,589,063

[45] Date of Patent: Dec. 31, 1996

[54] **COLUMN ANALYZER SYSTEM AND IMPROVED CHROMATOGRAPH COLUMN FOR USE IN THE SYSTEM**

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[73] Assignee: **Helena Laboratories Corporation**, Beaumont, Tex.

[21] Appl. No.: **470,991**

[22] Filed: **Jun. 6, 1995**

Related U.S. Application Data

[62] Division of Ser. No. 285,014, Sep. 16, 1994, Pat. No. 5,441,645, which is a division of Ser. No. 58,364, May 10, 1993, Pat. No. 5,358,641, which is a division of Ser. No. 779,186, Oct. 22, 1991, Pat. No. 5,228,988, which is a continuation-in-part of Ser. No. 733,714, Jul. 22, 1991, abandoned, which is a continuation-in-part of Ser. No. 708,298, May 31, 1991, Pat. No. 5,207,918, which is a division of Ser. No. 427,346, Oct. 27, 1989, Pat. No. 5,045,208.

[51] **Int. Cl.**[6] **B01D 15/08**

[52] **U.S. Cl.** **210/198.2**; 210/656; 422/70; 436/161; 436/809; 73/61.53; 73/61.55; 73/61.56

[58] **Field of Search** 210/198.2, 656; 422/70; 436/161, 809; 55/386; 73/61.52, 61.53, 61.55, 61.56

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Primary Examiner—Neil McCarthy
Attorney, Agent, or Firm—Dorsey & Whitney LLP

[57] **ABSTRACT**

An analyzer system for automatic column chromatography includes chromatographic columns which are mounted in a rack. A drop removal mechanism system shakes the rack to transfer the last drop of eluate from the tip of a chromatographic column to a receptacle below the tip before the column is repositioned over another receptacle and another fluid or operation is introduced into the column. This prevents one eluate from contaminating another. The chromatographic column may have an upper end which is sealed by a foil member. During use, the foil member is ruptured by lowering a pressure cylinder with a seal-punching head against it. The pressure cylinder, which is part of the pressure tip unit, is then withdrawn so that fluid can be introduced into the column via the ruptured foil member. The pressure tip unit is then lowered into sealing engagement with the column so that pressure can be applied via a bore in the pressure cylinder. The column may also have a tip portion which is originally sealed by a removable cap. The rack has a plate with aperture that is configured to receive the tip portion of the column and to provide a bearing surface for a spring member which removes the cap. This spring member is lowered through the aperture, and the bearing surface forces it toward the tip portion so that the cap can be pushed off.

24 Claims, 11 Drawing Sheets

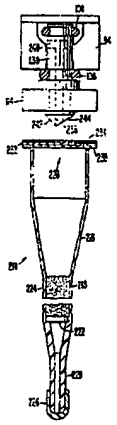

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FIG. 1

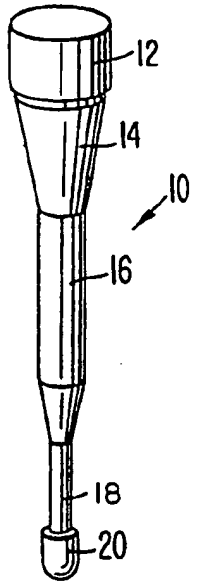


FIG. 2

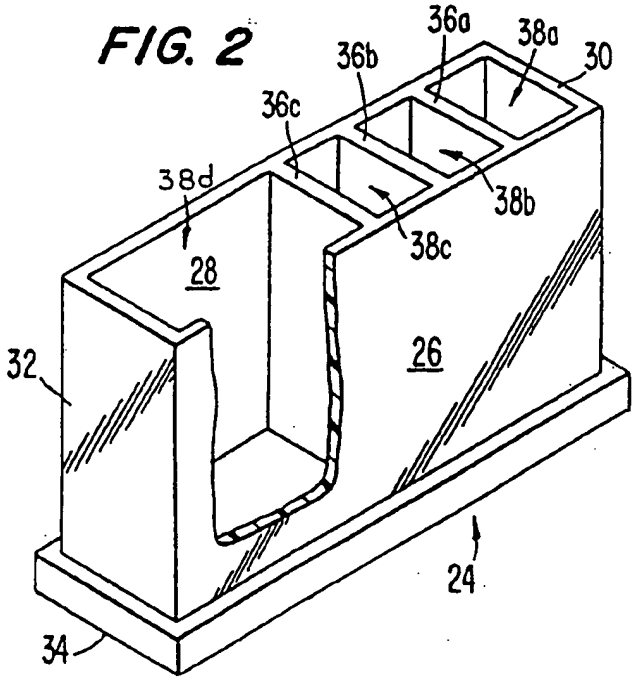


FIG. 3

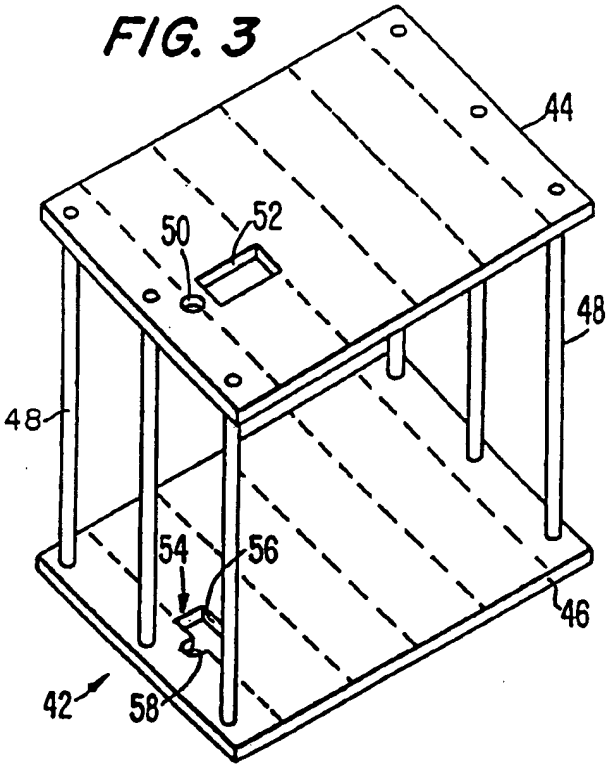


FIG. 4

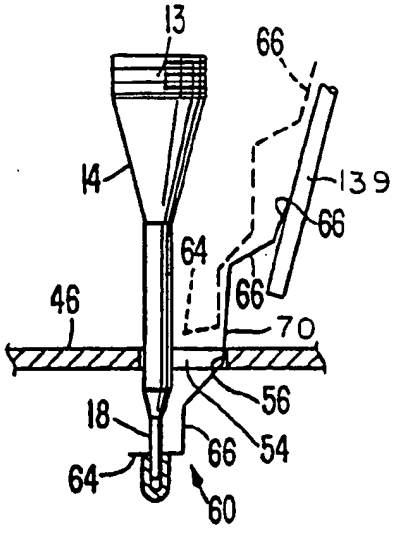


FIG. 5A

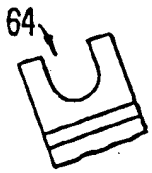


FIG. 5B

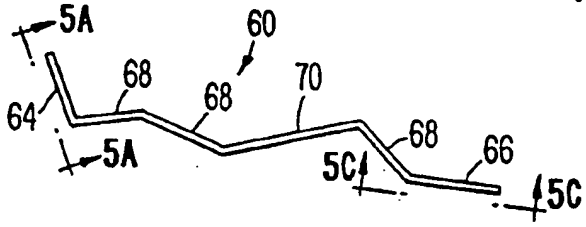


FIG. 5C

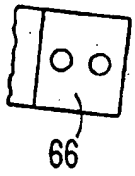
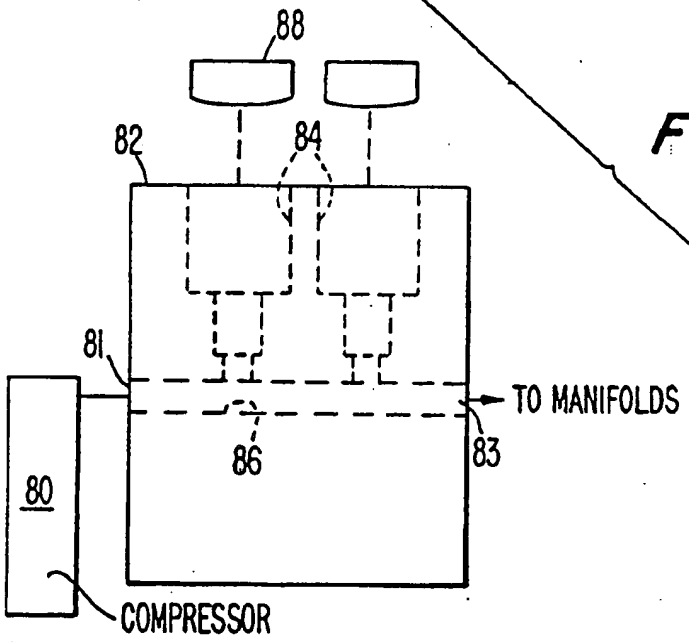


FIG. 6



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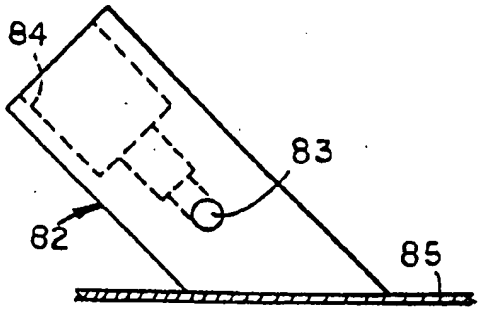


FIG. 8

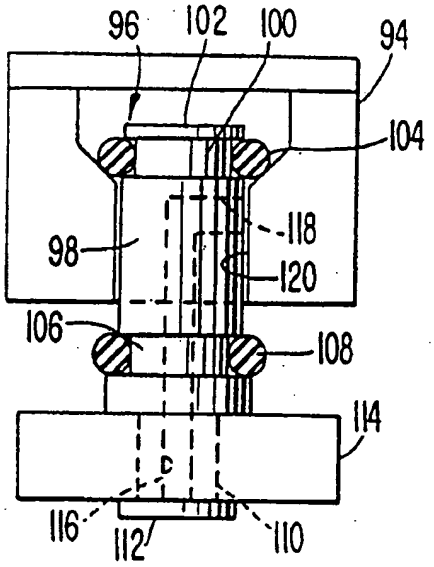


FIG. 9

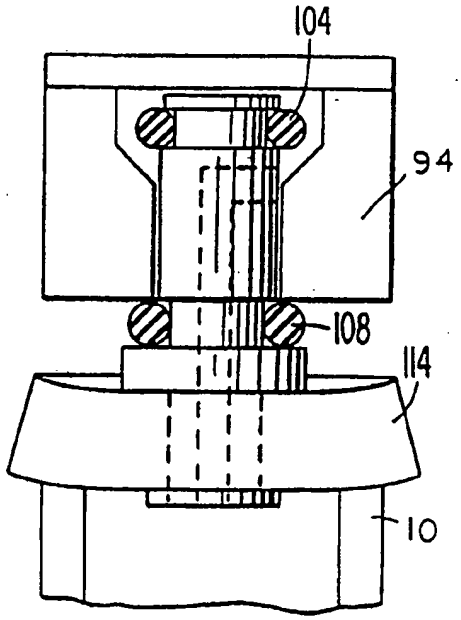
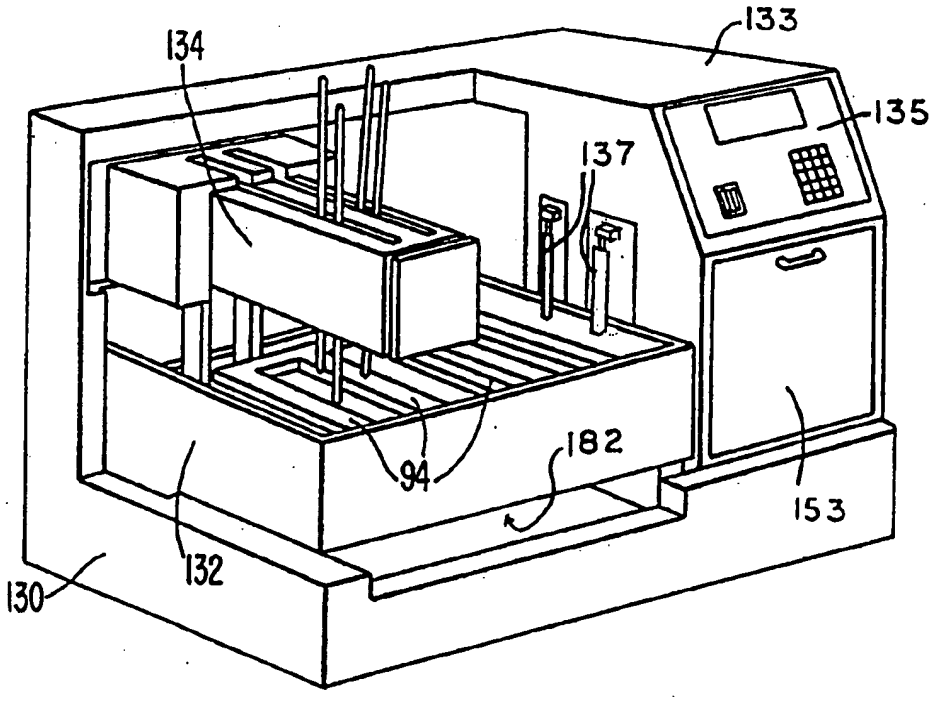


FIG. 10



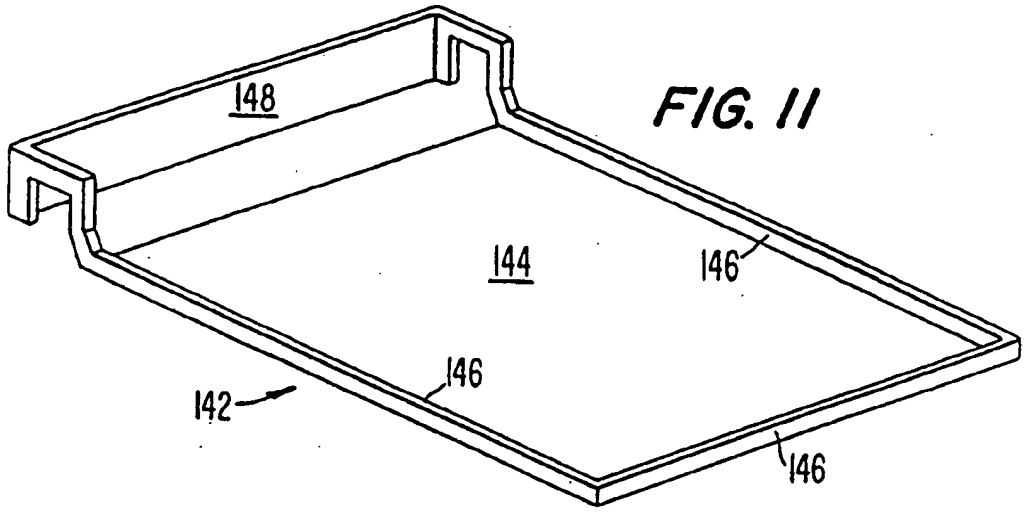


FIG. 11

FIG. 14

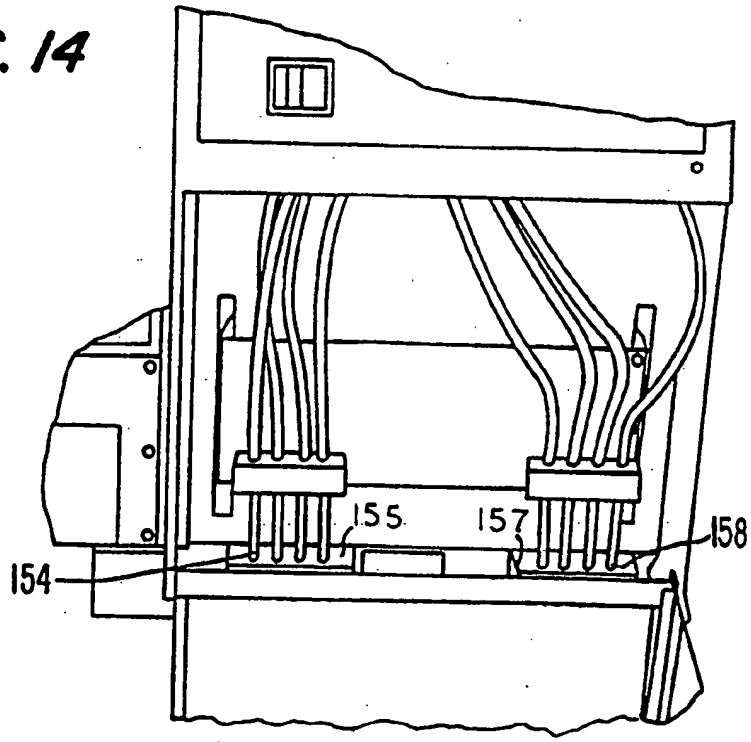


FIG. 12

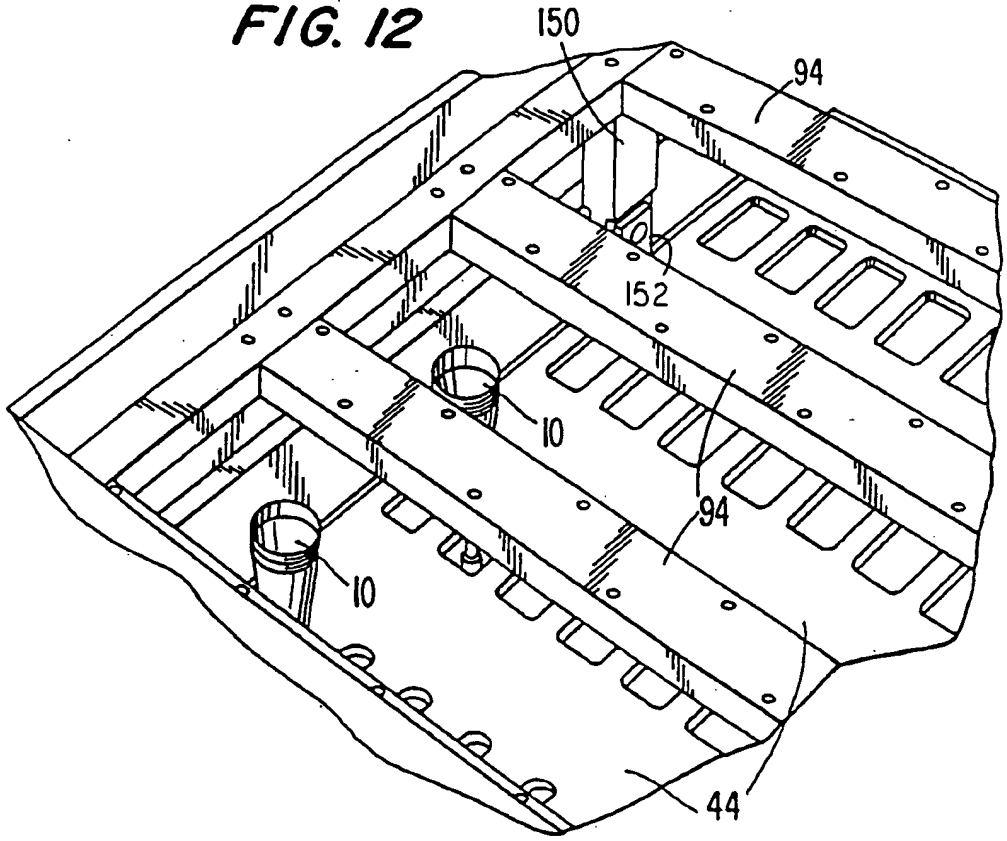


FIG. 13

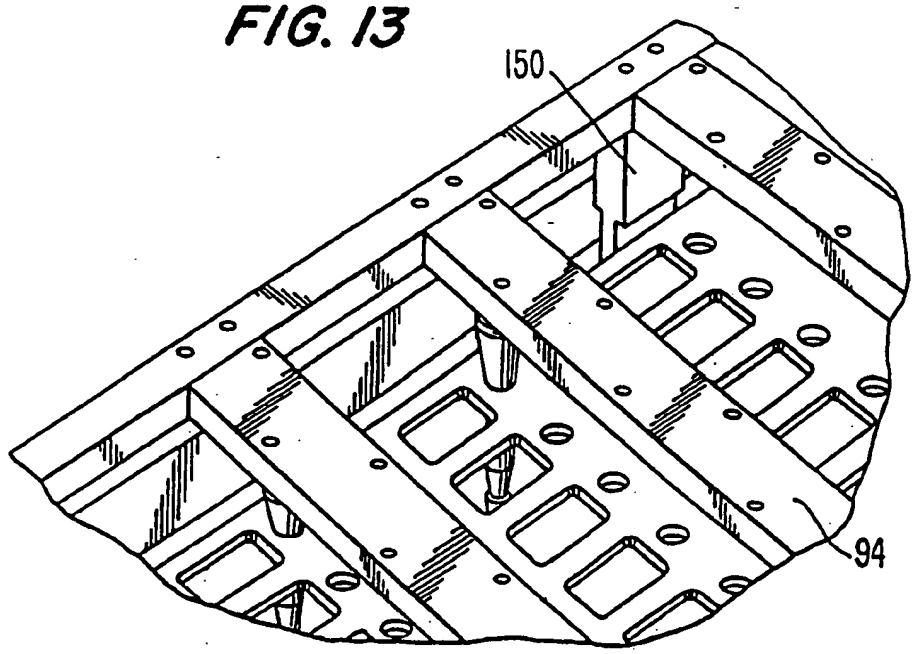


FIG. 15

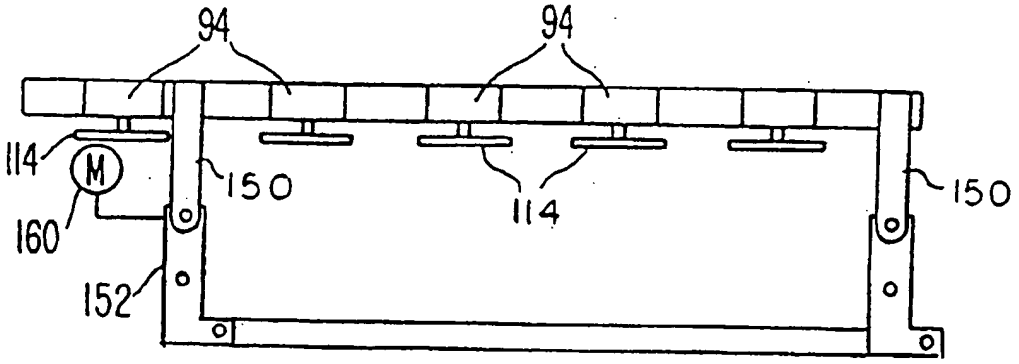
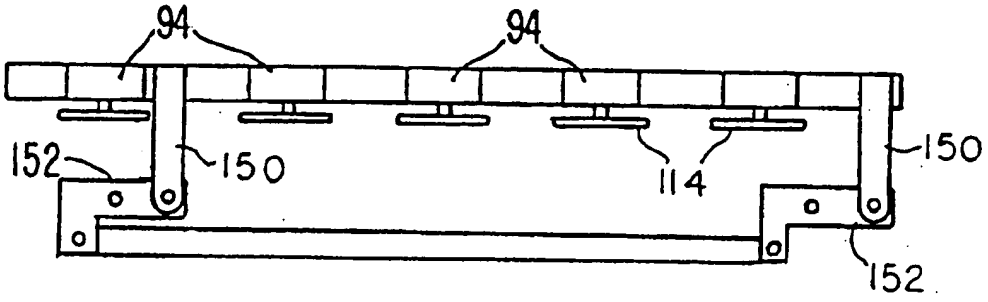


FIG. 16



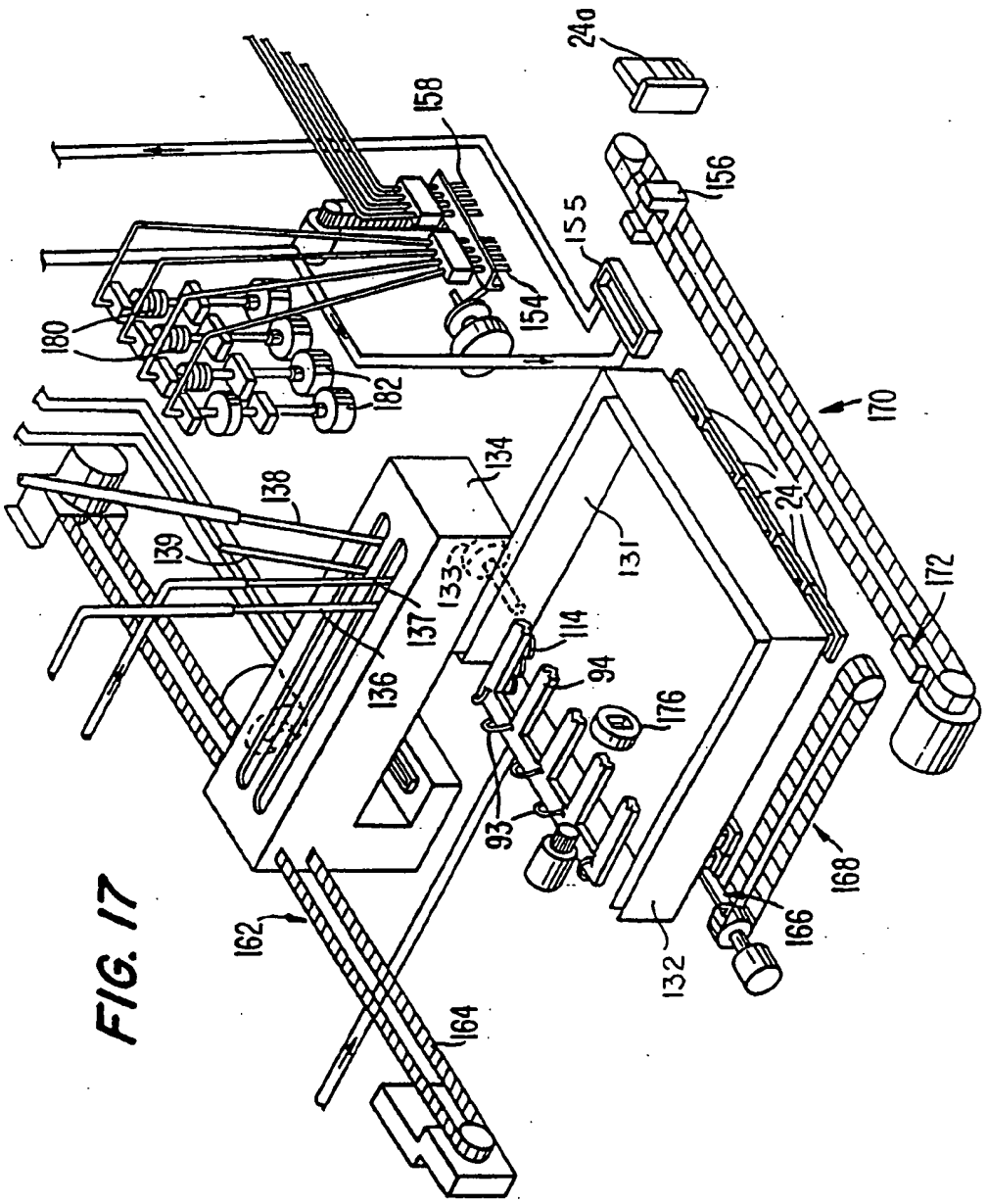

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FIG. 17

FIG. 18

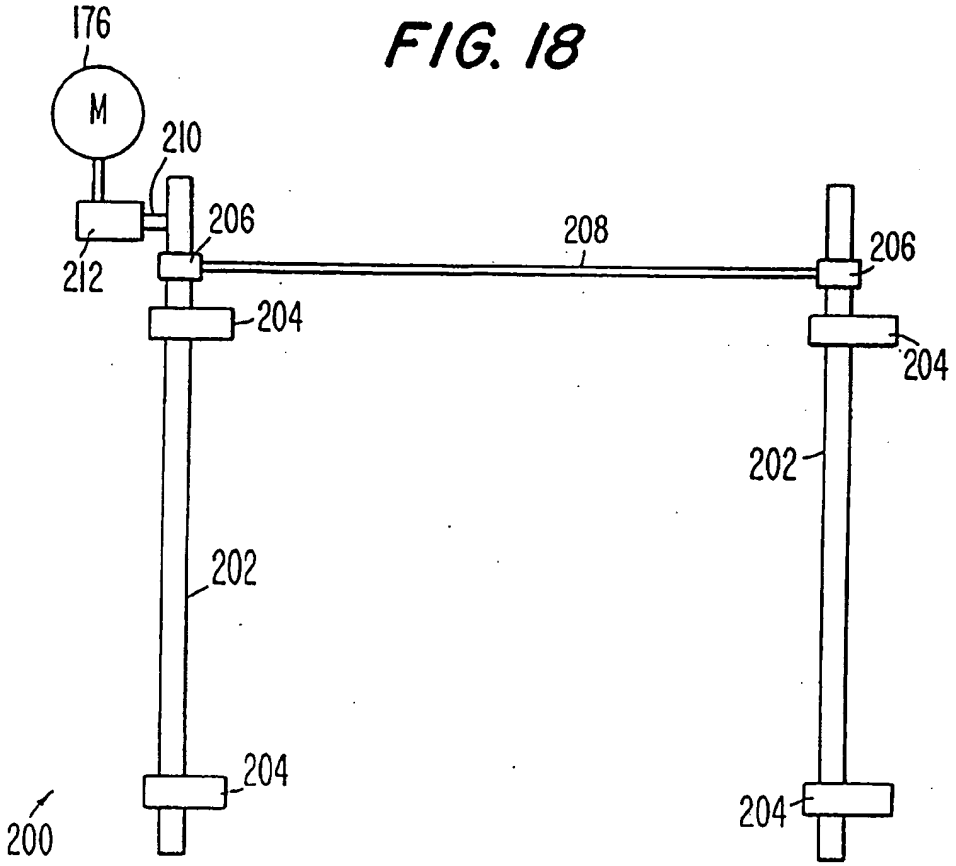
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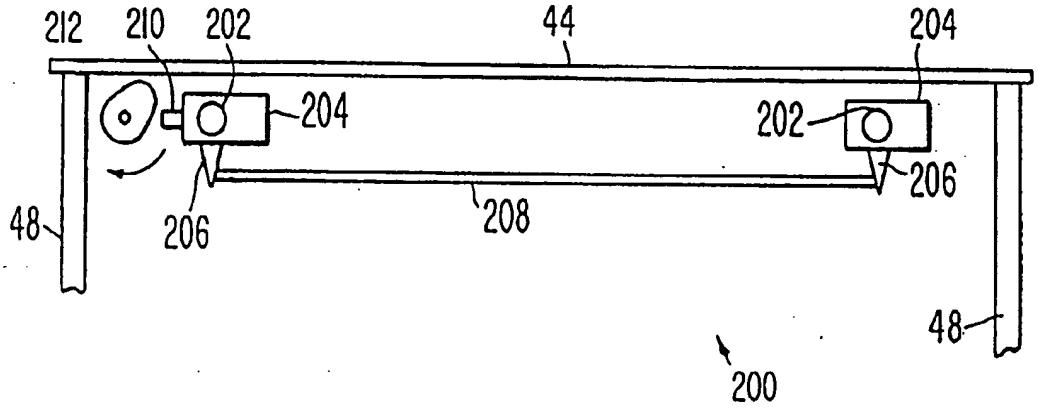
FIG. 19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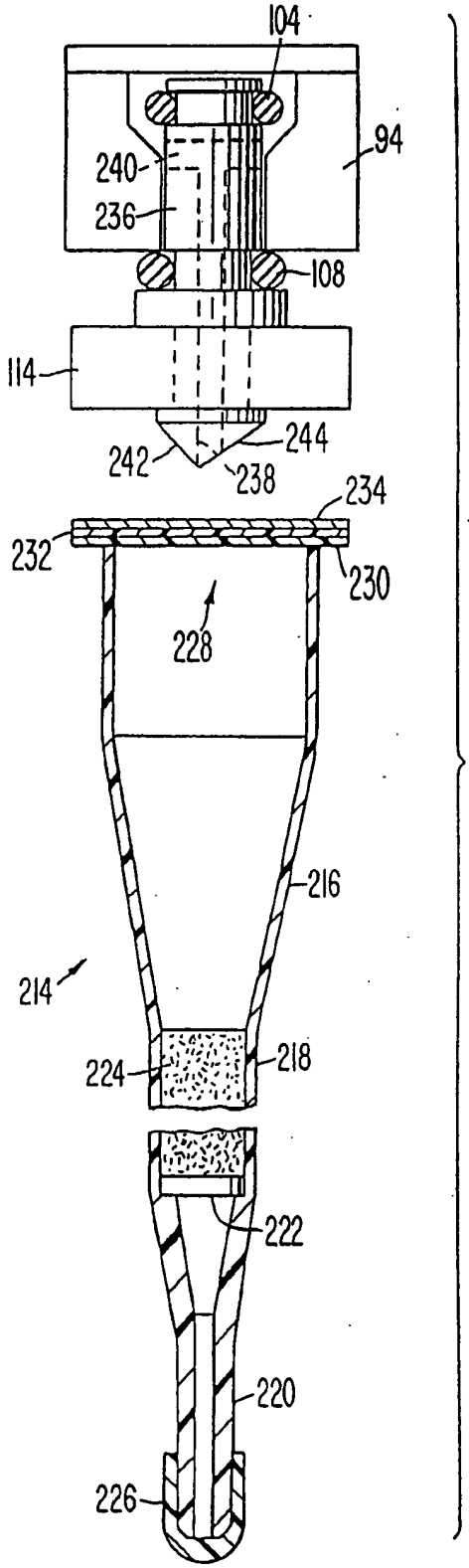
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FIG. 20

FIG. 21B

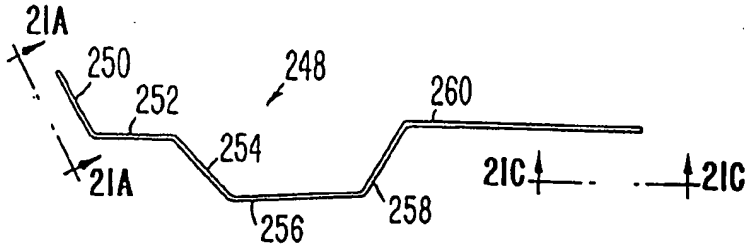

FIG. 21A

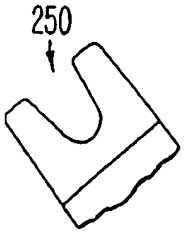

FIG. 21C

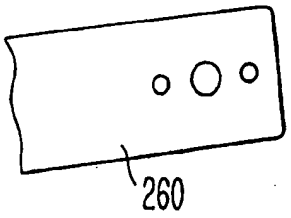

FIG. 23

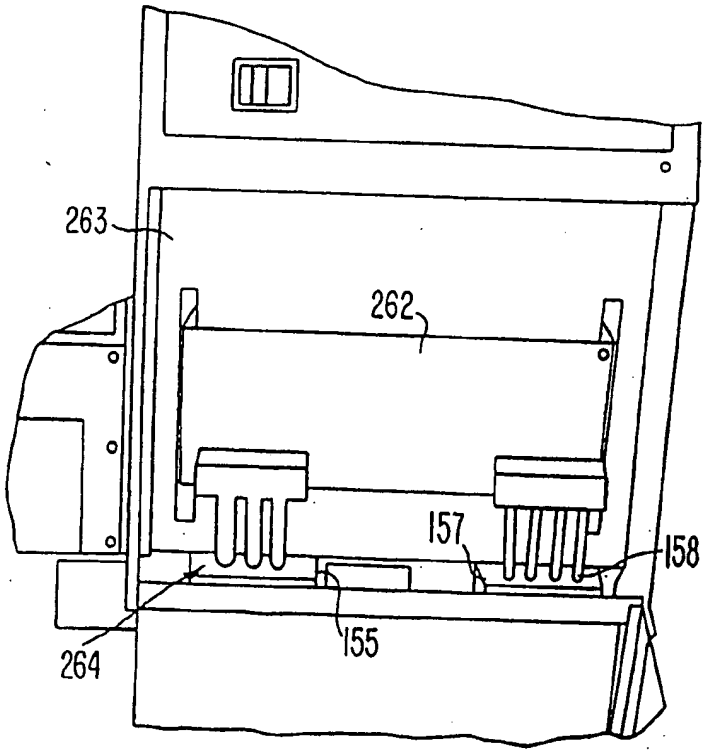

FIG. 22

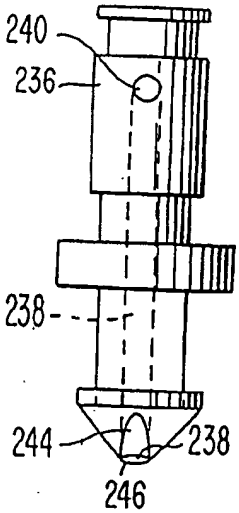

FIG. 25

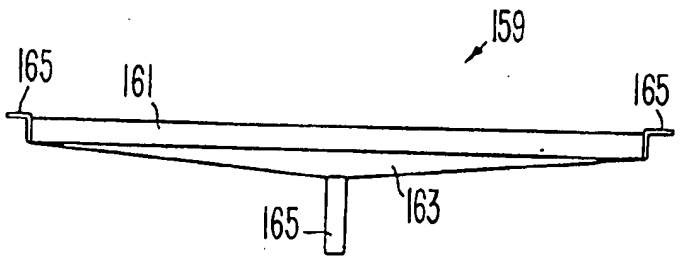
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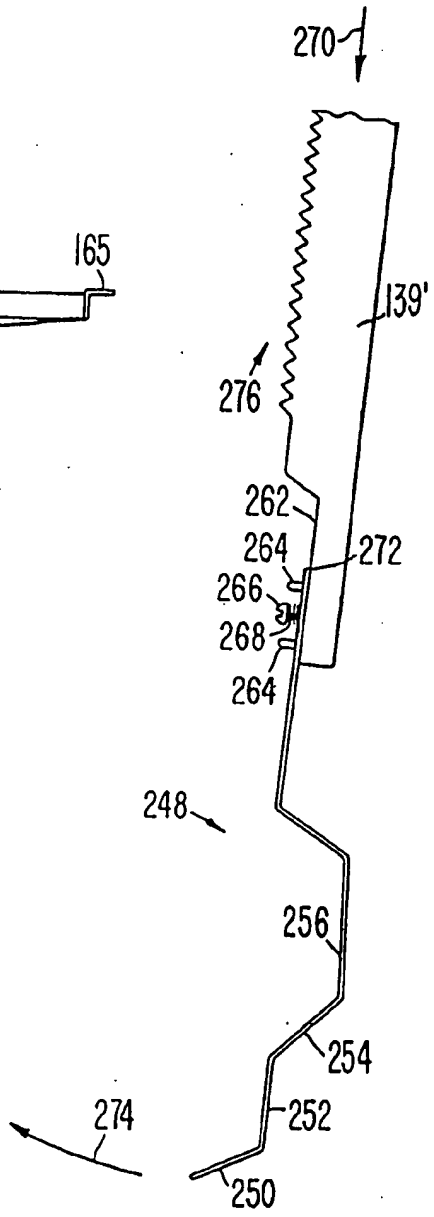
FIG. 24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COLUMN ANALYZER SYSTEM AND IMPROVED CHROMATOGRAPH COLUMN FOR USE IN THE SYSTEM

This is a division of application Ser. No 08/285,014 filed Sep. 16, 1994, now U.S. Pat. No. 5,491,645 which is a division of application Ser. No. 08/058,364 filed May 10, 1993; now U.S. Pat. No. 5,358,641 which is a division of application Ser. No. 07/779,186, filed Oct. 22, 1991, now U.S. Pat. No. 5,228,988; which is a Continuation-in-Part of application Ser. No. 07/733,714, filed Jul. 22, 1991, now abandoned; which is a Continuation-in-Part of application Ser. No. 07/708,298, filed May 31, 1991, now U.S. Pat. No. 5,207,918; which is a division of application Ser. No. 07/427,346, filed Oct. 27, 1989, now U.S. Pat. No. 5,045,208.

BACKGROUND OF THE INVENTION

This invention relates to column chromatography and, more particularly, to an improved column analyzer system which provides for automatic column chromatography and automatic optical density processing.

Column chromatography, using chromatographic media contained in small disposable columns (often called micro-chromatography), is a well-known technique utilized as part of clinical chemistry for analyzing various constituents of biological fluids, such-as blood. These media may be prepared from cellulose, agarose, silica, and various plastics, and typically utilize the presence of attached groups which are ionic (cation and anion), hydrophobia, or affinity specific in character. For example, Helena Laboratories Corporation, the assignee of the present invention, has marketed equipment and accessories for column chromatography as well as photometers or optical readers. Patent literature relating to column chromatography includes, for example, U.S. Pat. No. 4,341,635, issued Jul. 27th, 1982 to Tipton Golias and assigned to Helena Laboratories Corporation (as well as the prior art cited therein), and there is commercially available equipment relative to automating one or more aspects of column chromatography. For example, at least one machine has been marketed prior to the present invention which automates the introduction of fluids into a chromatographic column, and the-collection of the eluates from the column. Such equipment also provides for the processing of a plurality of chromatographic columns which are arranged in an array or matrix within the equipment.

The prior art, however, suffers from numerous disadvantages and shortcomings relative to the safe, accurate and expeditious chromatographic processing and subsequent optical density readings.

For example, according to the prior art, the eluate solutions are collected in a series of cuvettes, one cuvette for each eluate solution or fraction. Thereafter, the cuvettes are sequentially processed in an optical reader. This technique is time consuming, permits errors in identification of the cuvettes and exposes the technician handling the cuvettes to biological hazards such as HIV virus which may be carried in the blood specimens. The equipment which automates the processing of columns has heretofore discharged the eluates into a series of test tubes, and then the technician or operator of the equipment must manually transfer the test tubes to a reader.

While extremely high pressure liquid chromatography (HPLC) is well known, and while pressurized chromatography is known based upon the aforementioned Golias U.S. Pat. No. 4,341,635, we have discovered that a constant low pressure greatly enhances liquid chromatography. Prior to the present invention, however, constant low pressure liquid chromatography was not available.

SUMMARY OF THE INVENTION

The present invention overcomes the shortcomings of the prior art by providing a new and improved column analyzer system and method.

The column analyzer system of the present invention provides for the automated processing of an array of chromatographic columns, including the rupturing of foil seal members from the columns using pressure tip units with seal-punching heads and the removal of caps from the columns using spring members which are deflected by bearing edges provided by the racks in which the columns are mounted, without technician intervention solutions.

The present invention further provides for automatically shaking loose the last drop of eluate from a column to avoid transferring it to the wrong receptacle.

The present invention further provides for an improved chromatography column whose top end is sealed by a foil member.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing objects and advantages of the present invention, together with other advantages which may be obtained by its use, will become more apparent by reading the following detailed description of the invention taken in conjunction with the drawings.

In the drawings, wherein like reference numerals identify corresponding components:

FIG. **1** is a perspective illustration of a chromatographic column;

FIG. **2** is a perspective illustration, partially broken away, of a cuvette;

FIG. **3** illustrates, in perspective view, a rack for supporting a plurality of chromatographic columns;

FIG. **4** is a side view, partially in section, illustrating an embodiment of the apparatus for removing the caps from the chromatographic columns;

FIG. **5**, comprising FIGS. **5A**, **5B** and **5C**, illustrates the cap removal means in FIG. **4** with elevation views of each end thereof and with an edge view thereof;

FIGS. **6** and **7** illustrate the system for supplying constant, low pressure including a pressure regulator;

FIG. **8** illustrates an embodiment of the pressure tip associate with a single chromatographic column in a first position disengaged from a column;

FIG. **9** illustrates the pressure tip associated with a single column in the engaged position;

FIG. **10** illustrates, in perspective view, the apparatus of the present invention;

FIG. **11** illustrates, in perspective view, a tray for supporting the cuvettes;

FIG. **12** illustrates a portion of the top of the column rack and a portion of the manifold system in a disengaged position as seen from the back of the apparatus;

FIG. **13** illustrates the top of the column rack and a portion of the manifold system of FIG. **12** in an engaged position;

FIG. **14** is a partial front illustration with the door of the apparatus removed;

FIG. 15 is a diagrammatic illustration of the motor and linkage for positioning the manifold system in the disengaged or upper position;

FIG. 16 is a diagrammatic illustration of the motor and linkage for positioning the manifold system in the engaged or lowered position;

FIG. 17 illustrates, diagrammatically, the apparatus of the present invention including, in general terms, the location of various fluid supplies and the location of the optical density reader.

FIG. 18 is a top view schematically illustrating the drop removal mechanism;

FIG. 19 is a front view of the drop removal mechanism, and shows its cooperation with the top plate of the rack for holding the chromatographic columns;

FIG. 20 illustrates a chromatographic column sealed by a foil member in accordance with an embodiment of the present invention, along with a modified embodiment of a pressure tip unit for use with the column;

FIG. 21, comprising FIGS. 21*a*, 21*b*, 21*c*, illustrates a modified embodiment of the cap removal means with elevation views of each end thereof and with an edge view thereof;

FIG. 22 is a side view of the pressure cylinder in the pressure tip unit of FIG. 20;

FIG. 23 is a partial front illustration with the door of the apparatus removed, and shows a modification of part of the structure depicted in FIG. 14.

FIG. 24 is a side view illustrating the modified embodiment of the cap removal means mounted on an actuating mechanism; and

FIG. 25 is a front plan view of a tray that can be used during column regeneration.

DETAILED DESCRIPTION OF THE INVENTION

With reference to the drawings, FIG. 1 illustrates a chromatographic column 10 which includes an upper end or cover 12 threadingly engaged onto the body portion of the column. The body portion of the column includes a downwardly and inwardly tapering reservoir portion 14 communicating with a barrel portion 16, which barrel portion contains a chromatographic medium, such as affinity resin particles, cellulose, ion exchange resin particles, etc. The lower end of the barrel portion 16 tapers downwardly to a tip portion 18 which is covered by a removable cap 20. The column 10 as described and illustrated, including a threadable cover 12 (threads 13 are shown in FIG. 4), is conventional.

During liquid chromatography, it has been conventional, prior to the present invention, for the eluates to be discharged into a series of cuvettes or a series of test tubes. When test tubes are used, the contents may subsequently be transferred into cuvettes, depending on the equipment employed. The body of a test tube tends to interfere with the optical density reading, and a cuvette, with its generally flat walls, avoids this problem. However, prior to the present invention, each of the eluates from a chromatographic process have been collected in discrete cuvettes leading to the risk of errors in the processing of the individual cuvettes.

The present invention provides an improved cuvette means 24 which provides for the collection of all of the eluates from a single column within separate cells of a single cuvette means. With reference to FIG. 2, the cuvette means 24 is a generally rectangular container having opposed, parallel, spaced-apart side walls 26, 28, and opposed, spaced-apart parallel end walls 30, 32 oriented perpendicular to the side walls. The side walls and end walls of the cuvette means are mounted on a base 34, and the side walls and end walls, together with the base, define a rectangular container which is open at the top and closed at the bottom.

By way of example, but not by way of limitation, the cuvette means may include four separate cells. Three interior walls 36*a*, *b*, *c* extend between the side walls 26, 28, with the interior walls being generally parallel to the end walls 30, 32. The cuvette means thus defines four discrete cells 38 *a*, *b*, *c*, *d*, respectively. Each cell is defined by the base 34, the side walls 26, 28, and two additional walls. In the case of cell 38*a*, the two additional walls are walls 30 and 36*a*; for cell 38*b*, the two additional walls are walls 36*a* and 36*b*. The two additional walls which define cell 38*c* are interior walls 36*b* and 36*c*. The two additional walls which define cell 38*d* are walls 36*c* and 32. It should be noted that cells 38 *a*, *b*, *c* are of generally equal size and are substantially smaller than cell 38*d* for reasons which will be hereinafter explained.

Referring to FIGS. 3, a rack 42 is provided for supporting an array of columns. The rack includes upper and lower plates 44, 46, respectively, supported and maintained in spaced-apart relationship by a plurality of cylindrical rods 48. In the present embodiment of the invention, the upper and lower plates support 50 columns 10 in a 5×10 array. The upper plate 44 is provided with a series of circular apertures 50 of a size and shape to receive the barrel 16 of the column 10. Adjacent each circular aperture is a rectangular aperture 52 to accommodate the automatic column cap removal means which will hereafter be explained an to provide access to the cuvettes beneath rack 42. The lower plate 46 includes a plurality of apertures 54 which are generally rectangular in configuration. Each generally rectangular aperture 54 includes opposed short walls, a first longer wall interconnecting the short walls, and a second wall 58 opposed form the longitudinal wall 56, which second wall includes a cutout portion that is generally configured as three-quarters of a circle. The diameter of the cutout portion is configured to support the lower end of the barrel 16 of the column, and is aligned under the circular aperture 50 in the upper plate. The longitudinal wall 56 is a bearing surface for the automatic cap removal. In FIG. 3, only a single set of apertures in plate 44 is illustrated although 50 such sets, in a 5×10 array are contemplated. Similarly, only a single aperture 54 is illustrated in plate 46 although a 5×10 array is contemplated. The rack is positioned at a first station in the apparatus of the present invention. The apertures 54 permit access to the cuvettes under rack 42.

After the chromatographic columns are loaded into the rack and placed in position at the first station in the apparatus of the present invention, means are provided for automatically removing the cap 20 from the lower end of each column. FIG. 4 illustrates two positions of the cap removal means, an intermediate position illustrated by dashed lines and an active position illustrated by solid lines. It will be apparent that upper plate 44 has been omitted from the drawing in FIG. 4. The automatic cap removal means 60 is an elongated, thin, stainless steel spring strip extending generally vertically (or, more exactly, at 5.5° angle from the vertical) through the rectangular portions of the apertures 52, 54 in the upper and lower plates of the rack 42. As also illustrated in FIG. 5, the elongated, thin, steel spring includes a fork portion 64 at the lower end, an apertured upper end 66 for attachment to an actuating mechanism 139, and a series of intermediate portions 68 and 70 therebe-

tween, one of which intermediate portions bears against the bearing surface wall **56** as the cap removal means is moved vertically downward within the rack **42**. The cap removal means is attached to actuating mechanism **139** by screws (not shown) extending through the apertures in end **66**. As the actuating mechanism moves the spring **60** vertically downward, from a rest position above the column and past the intermediate position shown in dotted lines, the spring **60** engages the longitudinal wall **56** in the lower plate **46**. The continued downward movement of the spring against the bearing surface **56** urges the fork means toward the left to the active position illustrated in solid lines in FIG. **4**, and the tip **18** of the column is engaged between the fork tines. After portion **70** reaches bearing surface **56**, the continued downward movement of the spring forces the cap **20** downwardly until the cap is free of the column. The cap will, in practice, be retained in the first cell **38***a* of the cuvette means. Thereafter, the cap removal means is withdrawn vertically upwardly.

Means are provided for automating the column chromatography processing including such steps as applying hemolysate to the sample, agitating the column, running a buffer through the column, thereafter adding the sample to the column, adding the reagent to the column, etc. In general terms, this automatic technique is part of the prior art. However, the present invention includes certain features which are not found in the prior art. One of the these features is the provision of constant, low pressure for the chromatographic columns which are being processed. The pressure system will now be explained.

Referring to FIGS. **6** and **7**, the pressure system includes a compressor **80** for providing constant air pressure to an input port **81** of a regulator **82**. The regulator **82** also includes an output port **83**.

The regulator **82** includes two pressure relief bores **84**, each of which is generally T-shaped in cross section, and air from the compressor **80** flows through the input port **81**, through the main conduit **86** and through the output port **83** of the regulator. The two relief bores **84** are provided to permit operation of the regulator at two different constant pressures. For this purpose, each relief bore is provided with a projectile shape plunger **88**, the plunger in one relief bore being of a different weight than the plunger in the other relief bore. In addition, each relief bore **84** may be selectively closed at the exterior surface thereof such that only one relief bore will be in use at any given time. FIG. **7** illustrates the orientation of the regulator **82** when it is mounted on a chassis **85** during use. It will be noted that relief bores **84** are positioned at an angle relative to the vertical axis. In operation, with the plungers mounted within the respective relief bores and with one of the relief bores open and the other closed, if the air pressure through the conduit exceeds a threshold as determined by the weight of the plunger associated with the open relief bore, the air will move the respective plunger upward opening a fluid flow path through the relief bore thus providing for a bleed of air pressure. When sufficient air pressure bleeds out through the relief bore, the plunger drops back to seal the relief bore from the main conduit. Selector valves (not illustrated) permit pressure relief bores **82** to be selectively sealed from main conduit **86**. Positioning the plungers in bores **84** at an angle to the vertical facilitates movement of the plungers.

According to the principles of the present invention, a low but constant air pressure should be maintained at the output port **83**. The pressure that is selected should be high enough to increase the flow rate through the chromatographic columns but not high enough to compress the resin beds of the columns or to allow the fluid in the beds to be expelled. The optimum pressure will depend on the properties of the columns and the resin matrixes therein. For example, a pressure of about three inches of water has been found to be appropriate for columns with a cellulose resin available from Whatman Incorporated, while a pressure of about eight inches of water has been found to be appropriate for columns with "Emberlite" CG50 resin, available from Rohm & Haas. The weights of the two plungers may be selected such that one plunger is sufficiently light so as to permit a constant air pressure equivalent to three inches of water (for example), and the second plunger is slightly heavier, thus permitting a constant air flow pressure at eight inches of water (for example). It should be further appreciated that the low pressure employed is not limited to the range of three-eight inches of water, which is merely illustrative. Less than three inches or more than eight inches may be utilized as the constant low pressure, depending on the particular chromatographic columns that are used, while achieving the objective of the present invention.

Experience has shown that regulator **82** can be simplified if a quality piston compressor is used for compressor **80**. Screw-adjustable needle valves (not shown) can then be used in lieu of plungers **88** to provide air at a fairly constant low pressure to the columns.

Referring next to FIG. **8**, it should be remembered that the columns are provided in an array, heretofore described as a 5×10 array, namely, 10 chromatographic columns in each of 5 rows. The output from the pressure regulator **82** is coupled through tubes **93** (see FIG. **17**) to each of five manifolds **94**, each associated with one row. Each manifold **94** is connected to a pressure tip system of the present invention for providing the desired pressure to each of the chromatographic columns while, at the same time, preventing leakage of air if less than a full array of columns is being processed by the system. The pressure tip system, as illustrated in FIG. **8** in the absence of a chromatographic column, includes an upper block or manifold **94** having a conduit **96** therethrough in fluid communication through tube **93** with the output **83** of the pressure regulator. Mounted partially within the block **94** is an elongated pressure cylinder **98**, of generally circular cross section, having three reduced diameter circumferential recesses machined therein. Specifically, cylinder **98** has an upper grove or recess **100** machined therein, the upper groove positioned inwardly of a flange **102**. An O-ring seal **104** is positioned within the groove **100**. A second groove **106** is provided intermediate the two ends of the cylinder **98**, and an O-ring **108** is positioned in the second groove **106**. A third groove **110** is provided in the cylinder adjacent the second end and is spaced inwardly therefrom such that a lower flange **112** is provided at the second end of the cylinder **98**, and a flexible, foam gasket **114** is provided and mounted in the groove **110**, the foam gasket **114** being retained by the flange **112**. The cylinder **98** has a longitudinal bore **116** extending through the flange **112** and through the body of the cylinder, the bore **116** extending along the longitudinal center line of the cylinder of the upper groove **100**. A transverse bore **118** is provided generally perpendicular to the longitudinal bore **116** and intersecting the longitudinal bore **116** at the end thereof inwardly of the upper groove **100**. The longitudinal bore **118** is in fluid communication with a circular passageway **120** which is provided within the block **94**.

In the absence of a chromatographic column, the pressure tip system is positioned as illustrated in FIG. **8** such that the weight of the pressure tip system pulls the cylinder vertically downwardly causing the O-ring **104** to seal the top of the

passageway **120** from the conduit **96**, thus preventing the air flow from entering the vertical passageway **120**. A spring (not illustrated) can also be used above flange **102** to urge pressure cylinder **98** downward. Thus, O-ring **104** functions both as a seal and also as a retainer which engages the flange **102** and prevents the cylinder mechanism **98** from dropping downwardly out of the passageway **120** of the block **94**.

During the operation of the system of the present invention, the entire pressure system is movable such that cuvettes may be placed in the chassis, and chromatographic columns may be placed in frame **42** within the chassis. Thereafter, the pressure system is moved into position relative to the chromatographic columns and lowered into position such that for each chromatographic column mounted in the rack **42**, the top end of the column **10** engages the underside of the foam gasket **114** and pushes upward on the foam gasket such that the foam gasket, while still mounted in the groove **110**, moves the cylinder **98** upwardly until the O-ring **108** seals the vertical passageway **120** at the bottom of the block **94**. This is illustrated in FIG. **9**. Simultaneously pressure between the top of the column **10** and the underside of the foam gasket **114** provides an air-tight seal at the top of the column. Lastly, the vertical upward movement of the cylinder **98** moves the upper O-ring **104** clear of the top of the passageway **120**. Thus, air pressure through the conduit **96**, which is in communication with the output port **83** of the pressure regulator **82**, flows through the conduit **96** (for each pressure tip unit), downwardly through the passageway **120** and through the horizontal bore **118** and the vertical bore **116** and thereafter into the top of the chromatography column. A pressure system such as illustrated in FIGS. **8** and **9** is associated with each location in the array of columns.

Thus, it may be appreciated that if a full array of columns is being processed, each column presses upwardly on its respective foam gasket **114** to permit air pressure to flow into the top of the chromatographic column whereas in each position within the array, which is characterized by the absence of a chromatographic column, the pressure system remains in the position of FIG. **8**, and no air enters the cylinder **98** associated therewith.

Referring next to FIG. **10**, a perspective illustration of the apparatus of the present invention is illustrated. The apparatus of the present invention includes a chassis or frame **130**. The frame or rack **42** for the chromatographic columns is mounted within a housing **132** to the frame. FIG. **10** also illustrates the five manifolds **94** positioned above the rack **42**, the five manifolds or blocks extending from the front to the rear of the housing **132**. The cuvettes are positioned below the housing **132** as will be described hereinafter. At its right side, with respect to FIG. **10**, the apparatus includes a control housing **133** with a control panel **135**. Although not shown, a printer is disposed in control housing **133** to print out test results, and a sample rack is mounted on the front wall of housing **132** to hold small cups containing specimens that are to be transferred to the chromatographic columns.

Above the housing **132**, a horizontal support block **134** is mounted for movement laterally, i.e., in the "X" direction. Thus, the block **134** moves left and right. Mounted within the block are three discharge needles **136**, **137**, and **138**, along with actuating mechanism **139** (see FIG. **17**). The needles and actuating mechanism move as a group in the "Y" direction, i.e., forwardly and rearwardly of the block **134**. They also move in "Z" or vertical direction. Syringe pumps **137** are mounted on housing **133** and connected via tubing, not illustrated in FIG. **10**, to two of the needles. The syringe pumps transfer the samples and various fluids known in the art to the chromatographic columns. A tray **142** for supporting the cuvettes is slidably mounted under the housing **132** (see FIG. **11**).

FIG. **11** illustrates, in perspective, the tray **142** for the cuvettes, the tray including a generally flat surface or floor **144** upon which the cuvettes are placed, the floor being provided with guide rails **146** on three sides thereof. A handle **148** is provided on the tray for movement of the tray of cuvettes into and out of the apparatus of the present invention. The tray of cuvettes has been removed from FIG. **10** for the purposes of clarity and ease of illustration.

Experience has shown that technicians who place the cuvettes **24** on tray **142** may occasionally, through carelessness, set the base **34** (see FIG. **2**) of one cuvette on top to the base **34** of another cuvette near its edge, thus tilting the cuvette. Although not shown, this problem can be eliminated by attaching wedge-shaped spacers at the centers of the side walls **26** and **28** of the cuvettes and by altering the end walls **30** and **32** so that they have wedge-shaped cross-sections, which, in effect, provides spacers at the ends.

FIG. **12** illustrates, in perspective form, a rear view portion of the apparatus of the present invention, including the movable pressure tip system for engagement with the chromatographic columns. In FIG. **12**, three of the manifolds of blocks **94** are visible, as is the upper plate **44** of the rack **42** with two chromatographic columns **10** in place, one in each of two rows. At the upper right-hand corner of FIG. **12**, part of an L-shaped link **152** (also see FIGS. **15** and **16**) is visible. It is pivotably connected to a leg **150** which is attached to the manifolds **94**. Four such legs **150** are provided to support the manifolds, and each is pivotably connected to a respective link **152**. When the system is in the position illustrated in FIG. **12**, the links **152** are disposed in an upright position and the manifolds **94** are in their raised positions, offset from the columns **10**. In this raised and offset position, the needles **136**, **137**, **138** can access the tops of each of the chromatographic columns so that resuspension, separation, regeneration, or other fluids may be introduced therein or so that resuspension or other operations can be conducted.

FIG. **13** is a perspective illustration similar to FIG. **12** except that the links **152** have been rotated and the manifolds are now in their lowered position. When the links **152** are rotated from their upright positions, there is a downward swinging movement of the manifolds **94** such that the manifolds **94** move above the columns **10** and the pressure tip system of FIGS. **8** and **9** makes contact with the tops of the columns.

FIG. **14** illustrates an enlarged, partial front elevation view of the apparatus of the present invention with the door **153** of control housing **133** (FIG. **10**) open. In this position, a first group of mixing needles **154** is illustrated. There are four such needles, which can be associated with any cell of a cuvette. After chromatographic separation, the cuvette is moved to the right in FIG. **14** by a stepping motor and is held in position underneath the needles **154** such that the fluid within each cell may be thoroughly mixed. The cuvette is then moved further to the right in FIG. **14** to be scanned by an optical reader **156** (see FIG. **17**). After scanning by the optical reader **156**, the cuvette advances further to the right under a second group of needles **158** which remove fluid from the cuvette cells.

It was previously indicated that cuvette cell **38*d*** is larger than the other three cuvette cells. This is because in column chromatography, it is common to collect eluates and, in a separate container, dilute a second sample (e.g., of blood or other biological fluid) such that comparative optical density

readings may be taken for providing a reference standard for the purpose of quantifying purified fractions thereof relative to the standard. This technique is, of course, well known.

FIG. **15** illustrates, in diagrammatic form, linkage and a motor **160** for moving the manifold system to the raised position, in which the manifold system is retracted relative to the tops of the individual chromatographic columns such that reagents, and samples and the like may be introduced therein. As will be apparent, the L-shaped links **152** are in their upright position in FIG. **15**.

FIG. **16** illustrates, diagrammatically, the linkage and motor system of FIG. **15** in the engaged position, illustrating the 90° rotation of the aforementioned links **152**. It should be noted that while FIGS. **12** and **13** provide a rear perspective illustration, FIGS. **15** and **16** provide a front elevation diagrammatic illustration.

Referring next to FIG. **17**, an overall system illustration of the present invention will now be described. The rack **42** is attached to a frame **131** that is movably mounted within housing **132**. A motor **133** is provided to shift frame **131** and thus rack **42** laterally so that each column can be positioned above different cells. Furthermore, block **134** is illustrated as mounted to a stepping motor system **162** which moves the block **134** laterally through a drive belt **164**. The columns are processed from right to left and from front to back as illustrated in FIG. **17**. The cuvettes are processed from left to right and from back to front. To accomplish this, a bar or fence **166** extends across the width of the housing **132**, underneath the housing but above the tray **142**, and both ends of the bar are connected to a drive mechanism **168**. Advancing the drive mechanism **168** moves the bar **166** forwardly such that five cuvettes **24**, one from each row, move forwardly clear of the tray **142** and onto a drive mechanism **170**. Drive mechanism **170** incudes a pusher block **172** which advances the cuvettes sequentially underneath the mixer needles **154**, through the optical reader **156**, and thereafter underneath the evacuator needles **158**, which are connected through a pump to a waste evacuation system. After the five cuvettes are processed, the mixer needles **154** are cleaned in a wash tray **155** and the evacuator needles **158** are cleaned in a similar wash tray **157** (not shown in FIG. **10**). Drive system **168** advances the bar **166** forward, yet another step, such that the next row of five cuvettes may be deposited onto the drive belt **170** and processed sequentially. FIG. **17** also illustrates a cuvette **24***a* dropping off the drive mechanism after the completion of mixing, reading and evacuation.

After the last cuvette has been processed and removed from the apparatus, the technician attending the apparatus may remove all of the chromatographic columns **10** from the rack **44** and replace them with new ones in preparation for the next cycle of tests. However, economic circumstances may make it preferable to regenerate the columns **10** that have just been used instead. As is known in the art, during regeneration the columns are exposed to chemicals which restore the chromatographic medium in the columns **10** to its original condition. The column analyzer system is configured to permit regeneration without removing the columns **10** from the rack **42** if the columns are being regenerated for immediate re-use. Three regeneration techniques are particularly suitable.

In the first regeneration technique, the technician reloads tray **142** (FIG. **11**) with cuvettes **24**, and then reinserts the tray **142** in the opening **282** (see FIG. **10**) beneath housing **132**. The needles are then used to transfer a diluted acid to each of the columns **10**, followed by water. After the acid and water drip into the cuvettes **24** beneath the columns, perhaps hastened by applying a low pressure to the columns in the manner previously discussed, the tray **142** is removed and the cuvettes are emptied into a waste receptacle. Fresh cuvettes that will be employed during the next testing run are loaded onto tray **142**, which is then inserted into opening **282** before the next testing run is begun.

As an example, assume that the columns **10** have an affinity-type chromatographic media and have just been used during tests in which the specimens were blood. In this situation the chromatographic medium in the columns is contaminated with sorbitol, a type of sugar, which must be removed to restore the medium. The sorbitol can be stripped away using five milliliters of a 0.1 molar concentration of hydrochloric acid, followed by 5 milliliters of water to flush out the acid. Hydrochloric acid is recommended, although other acids such as sulfuric acid, nitrite acid, or acetic acid may be used. The molar concentration of the acid may range from about 0.5 to about 0.05, with 0.1 being recommended. Enough of the acid solution must be used in each column to remove the greatest possible deposit of sorbitol that might be encountered, and this must be followed by enough water to flush out the greatest acid residue that might remain.

The tray **142** is not used in the second of the regeneration techniques mentioned above. Instead, after tray **142** has been removed a modified tray **159** (see FIG. **25**) is inserted into opening **282** to collect the dilute acid and water which drips through the columns. Tray **159** is open at its top, and includes four walls **161** and a bottom **163**. A pipe **165** is connected to bottom **163** at a drain opening (not illustrated) in bottom **163**. Bottom **163** is shaped to slope slightly toward the drain opening. Rails **165** are attached to the two walls **161** at the sides of tray **159**. The rails **165** slide on corresponding rails (not shown) in the chromatographic apparatus when tray **159** is inserted into opening **182**. At the corners between wall **161**, tray **159** is sealed with a silicon-based sealant. Although not shown, pipe **165** is connected by tubing to a waste receptacle. After the regeneration procedure the dilute acid and water in the waste receptacle is emptied, and the modified tray is withdrawn from opening **282**. A tray **142** of cuvettes can then be inserted to begin a new testing run.

In the third regeneration technique mentioned above, a tray (not illustrated) corresponding to tray **159** is permanently mounted in the chromatographic apparatus, at a position below tray **142**. The acid solution and water can then drip into it when tray **142** is removed.

The above-noted regeneration techniques may be used even if the chromatographic columns **10** are to be removed from the system for use at a later date. In this case, after the regeneration procedure the attendant removes the columns **10** from rack **42**, installs caps **20**, pours in a preservative solution, and then applies caps **12**.

It should be further appreciated that as part of the present invention, stepping motor **176** (illustrated diagrammatically in FIG. **17**) is provided for driving a cam arrangement such that, at the completion of the chromatographic separation step, rotation of the motor causes raising and lowering the rack **42**. This movement causes any drops at the bottom of the tips of the chromatographic columns **10** to drop into the respective cells of the cuvettes **24**. This drop removal feature will be described in more detail with reference to FIGS. **18** and **19**.

A top view of drop removal mechanism **200** by itself is shown in FIG. **18**, and FIG. **19** illustrates a front view of mechanism **200** in its operative position beneath top plate **44**

of rack 42. Mechanism 200 includes a pair of rods 202 which are journaled for rotation beneath plate 44. Rods 202 extend parallel to manifolds 94 (see FIG. 10). Rectangular plastic blocks 204 are attached in an eccentric manner to the rods 202. A block 204 is positioned adjacent each corner of plate 44. Crank members 206 are also attached to bars 202, preferably at a position behind plate 44. Each end of a link 208 is pivotably attached to a crank member 206. Link 208 and crank members 206 cause rods 202 to rotate in unison. Drop removal mechanism 200 also includes a pin 210 affixed to one of the rods 202. Pin 210 is positioned for engagement by a cam 212 which is rotated by stepping motor 176.

Rack 42 normally rests on a support (not illustrated) but is mounted so that it can slide upward slightly from the support. When cam 212 undergoes a clockwise revolution (with respect to FIG. 19), it encounters pin 210 and causes rods 202 to rotate by about 90° before cam 212 separates from pin 210. During this 90° rotation, blocks 204 urge plate 44 and thus rack 42 upward about an eighth of an inch above the support. Rack 42 drops back to the support when cam 212 disengages from pin 210. The resulting jar is sufficient to jiggle loose any drops suspended at the end of the columns 10 supported by the rack 42.

Those skilled in the art will recognize that drop removal mechanism 200 improves the accuracy of the measurements made by the automatic column chromatography machine. During use of the machine fluids are introduced to each column 10 in a sequence, and the eluates from the column are collected in different cells of the cuvette means 24 (FIG. 2). Accuracy would suffer if even a small amount eluate intended for one cell were to contaminate the eluate collected in an adjacent cell. Accordingly, motor cell 176 is actuated to rotate cam 212 by a revolution after each solution has been introduced to the column 10, and before the column is advanced to the next position over an adjacent cell. The result is that the last drop falls into the cell intended instead of the adjacent cell.

The three needles 136, 137, and 138 and the actuating mechanism 139, illustrated in FIG. 10 and in FIG. 17, each move in the "Z" direction under influence of motors. The needles 136, 137, and 138 provide for mixing or agitation of the resin in the columns as well as providing conduits for the addition of reagents and/or samples into the columns. In addition, vertical movement of actuating mechanism 139 (also see FIG. 4) provides for removal of the caps from the columns. Actuating mechanism 139 functions to move spring 60 downwardly.

Reference should also be had to the bellows-motor 180 illustrated in FIG. 17 wherein four bellow systems are shown, each associated with one of the needles 154 and each attached to a motor 182. Motor 182 rotates, and an internal threaded nut system converts the rotation motion of the motor 182 into linear motion of the bellows 180 which, in turn, moves the fluid in the needles 154 vertically for mixing the liquid in the cuvette cells prior to the liquid passing through the optical reader 156.

The bellows-motor arrangement illustrated with respect to the needles or mixer unit 154 may be replicated for movement of the needles 136–139 as they provide controlled, bi-directional movement in small increments.

FIG. 20 illustrates an alternate embodiment for the pressure tip units, and a modified chromatographic column with which it is used. The arrangement shown in FIG. 20 avoids the need to manually unscrew covers 12 (FIG. 1) when the columns are loaded into the rack 42.

In FIG. 20, an improved chromatographic column 214 in accordance with the present invention includes a polypropylene shell which defines a reservoir portion 216, a barrel portion 218 (shown broken away to reduce its length in the drawing), and a tip portion 220. A fluid-pervious disk 222 lodged inside the shell supports a chromatographic medium 224 within barrel portion 218. The medium may be, for example, ion exchange resin particles, affinity resin particles, size-exclusion resins, or matrix-type media such as cellulose, agarose, or silica gel. A removable cap 226 closes the lower end of tip portion 216.

A foil seal member 228 closes the top end of reservoir portion 216. During fabrication of column 214, seal member 228 is fused to the shell using heat (alternatively, adhesive bonding or ultrasonic bonding can be employed). Member 228 includes an adhesion layer 230 of polyethylene which readily fuses with the plastic of the shell. Polypropylene could also be used in layer 230. Layer 230 is bonded to an intermediate layer 232, such as polypropylene. Polyethylene could also be used in layer 232. An aluminum layer 234 is bonded to 232. Layer 232 serves as a moisture barrier. The thickness of layers 230–234 is exaggerated in FIG. 20 for ease of illustration. Layers 230 and 232 are preferably a thousandth or so of an inch thick each. Layer 234 is less than about three thousandths of an inch thick, and preferably about one to two thousandths of an inch thick.

The pressure tip unit that cooperates with column 214 includes a pressure cylinder 236 having three reduced diameter circumferential recesses machined therein. Cylinder 236 extends movably through a bore in manifold 94. O-rings 104 and 108 are lodged in two of the recesses, and a foam gasket 114 is lodged in the third. An axial bore 238 communicates with a bore 240 across the diameter of cylinder 236. A seal-punching head 242 is provided at the bottom of cylinder 236.

During fabrication of pressure cylinder 236, head 242 is initially machined to be conical, with a 90° apex angle (that is, the surface has a 45° angle with respect to the longitudinal axis of cylinder 236). Then one side of head 242 is ground to provide a flat region 244. This leaves a cutting edge 246 (see FIG. 22) along one rim of the bottom opening of bore 238.

During operation up to 50 columns 214 are first loaded into the rack 42. Then motor 160 (FIG. 15) is actuated to move links 152 to the horizontal position as shown in FIG. 16 and thereby lower the manifolds 94. The cutting edges 246 of the pressure cylinders 236 pierce the aluminum layers 234 of the seal members 228, and the continued downward movement of the heads 242 ruptures the seal members 228. Motor 160 is then actuated again to move links 152 to their vertical positions as shown in FIG. 15. Fluids can then be added through the punctured seal members 228 before the manifolds 94 are lowered again to apply pressure to the columns in the manner previously discussed. The aluminum layer 230, being relatively thick, not only provides a secure closure during transportation and handling of the columns 214, it also prevents the ruptured seal member 228 from springing back after the pressure cylinder 236 has been withdrawn. That is, the aluminum remains permanently deformed and prevents the hole formed by head 242 from closing up.

Pressure cylinder 236 can of course replace pressure cylinder 98. An axial blind bore (not shown) can be drilled from the top to hold a spring (not shown) as an aid to closing pressure cylinder 98.

FIGS. 21A–21C illustrate a cap removal means 248 that can be used in lieu of the cap removal means 60 shown in

FIGS. 4 and 5A–2C. Cap removal means **248** includes a fork portion **250**, intermediate portions **252, 254, 256,** and **258,** and an end portion **260**. Cap removal means **248** is made from a stainless steel strip and cooperates with bearing surface **256** (FIGS. **3** and **4**) in the manner previously discussed with. respect to cap removal means **60**. However, while cap removal means **60** is flexed slightly and acts as a spring during operation, cap removal means **248** is substantially inflexible. Fork portion **250** is preferably 0.34 inches long and has a generally v-shaped slot with a rounded bottom. Intermediate portion **252** is preferably 0.40 inches long and forms an angle of 120° with fork portion **250**. Intermediate portion **254** is preferably 0.39 inches long and forms an angle of 135° with intermediate portion **252**. Intermediate portion **256** is preferably 0.66 inches long, and forms an angle of 132° with intermediate portion **254**. Intermediate portion **258** is preferably 0.37 inches long and forms an angle of 123° with intermediate portion **256**. End portion **260** is preferably 1.16 inches and forms an angle of 120° with intermediate portion **158**.

FIG. **24** shows cap removal means **248** attached to an actuating mechanism **139'** having a flattened lower end **262**. Pegs **264** are attached to lower end **262** and extend through the smaller openings in end portion **260** shown in FIG. **21C**. A screw **266** extends through the central opening, with the head of screw **266** being spaced apart from the surface of cap removal means **248**. A coil spring **268** is disposed around the shaft of screw **266** beneath the head of the screw, and normally biasses portion **260** against flattened lower end **262**. However, when actuating mechanism **139'** is moved downward in the direction of arrow **270** and portion **254** engages bearing surface **56** (see FIGS. **3** and **4**) of rack **42**, cap removal means **248** is pivoted about its upper end **272**, and fork portion **250** swings in the direction of arrow **274** toward the tip portion **18** (see FIGS. **1** and **4**) of a column **10**. During further downward motion, portion **256** slides against bearing surface **56** and the cap **20** at the tip of the column is dislodged. When actuating mechanism **139'** is later withdrawn, in the direction opposite arrow **270**, spring **268** urges cap removal means **248** back to its original position with respect to actuating mechanism **139'**. It will be apparent that pegs **264** serve to keep cap removal means **248** aligned, and that pegs **264** are not affixed to cap removal means **248** but are, instead, slightly movable with respect to it.

Spring **268** is shaped like a truncated cone, when seen from the side. Because of this, spring **268** can be flattened so that its coils are nested inside one another. Thus spring **268** can undergo considerably more compression than could a cylindrical spring. Spring **268** is preferably made of stainless steel, with a copper coating to prevent the stainless steel from gauling.

In FIG. **24**, actuating mechanism **139'** is shown with a rack of teeth **276** for engagement with a gear (not shown) rotated by a motor (not shown) in support block **134** (see FIGS. **10** and **17**) to raise or lower actuating mechanism **139'**. Actuating mechanism **139** (see FIG. **4**) may similarly be provided with a rack of teeth.

FIG. **23** shows a modification of FIG. **14** which avoids the need for the bellows **180** and motors **182** shown in FIG. **17**. In FIG. **23**, a plate **262** is mounted on a wall **263** so as to be movable up and down. Wall **263** is movable forward and backward. A paddle assembly **264** with three paddles is mounted on plate **262**. The paddles are lowered into cells **38***b*, **38***c*, and **38***d* of cuvette **24** and then moved forward and backward to mix the contents thereof. The paddle for cell **38***d* is larger than the rest since cell **38***d* itself is larger than the other cells. A fourth paddle is not needed since the content of cell **38***a* is not normally read- it contains the cap **20** and a buffer solution that was drained from the column before use. The needles **158** for evacuating the cuvettes are still used. However, the flexible hoses shown leading to the needles **158** in FIG. **14** are diverted behind plate **276** in the embodiment of FIG. **23**.

The foregoing is a complete description of a preferred embodiment of the present invention. The invention automates those steps normally performed by a technician in a manual pipetting operation, and the individual steps, per se, are conventional.

Many changes and modifications may be made without departing from the spirit and scope of the present invention. The invention, therefore, should be limited only by the following claims.

What is claim is:

1. A chromatographic column, comprising:

a hollow plastic shell having a reservoir portion with an end, a tip portion with an end, and a barrel portion connected between the reservoir and tip portions;

a chromatographic medium in the barrel portion; and

a foil member attached to the end of the reservoir portion to close it, the foil member having a metal layer and at least one plastic layer which separates the metal layer from the shell.

2. The column of claim **1**, wherein the foil member includes a plurality of plastic layers, and wherein the metal layer is thicker than any of the plastic layers.

3. The column of claim **1**, wherein the foil member comprises a polyethylene adhesion layer which is heat-fused to the shell, and a polypropylene intermediate layer which is bonded to the adhesion layer and to the metal layer, the metal layer being thicker than about a thousandth of an inch.

4. A method for conducting column chromatography, comprising the step of utilizing the chromatographic column of claim **1**, and further comprising the steps of:

(a) placing the chromatographic column in a rack;

(b) rupturing the foil member;

(c) introducing fluid into the column through the ruptured foil member; and

(d) pressurizing the interior of the column.

5. The method of claim **4**, further comprising the steps of placing a plurality of additional chromatographic columns with foil members in the rack during step (a); rupturing the foil members of at least one additional column during step (b); introducing fluid into at least one additional column during step (c); and pressurizing the interior of at least one additional column during step (d).

6. The method of claim **4**, further comprising the steps of collecting eluate from the column in a cuvette while step (d) is conducted, moving the cuvette to a measurement station after the eluate is collected, and sensing at least one property of the eluate at the measurement station.

7. The method of claim **4**, wherein a pressure cylinder has a bore, a conical portion that extends to the bore, a flat portion that extends to the bore, and a cutting edge adjacent the bore; and wherein step (b) comprises rupturing the foil member with the cutting edge of the protruding head.

8. A pressure cylinder for use in the method of claim **4**.

9. The method of claim **4**, further comprising the steps of collecting eluate from the column in a cuvette while step (d) is conducted, the cuvette comprising at least two cells for discrete retention of liquid therein.

10. The method of claim **9**, wherein the cuvette includes at least three cells for discrete retention of liquid therein.

11. A method for conducting column chromatography, comprising the steps of utilizing the chromatographic column of claim 1, wherein the tip portion is provided with a removable cap, and further comprising the steps of:

(a) positioning the chromatographic column in a support;

(b) lowering a decapper member into engagement with the tip portion to remove the cap from the tip portion of the column.

12. The method of claim 11, further comprising the steps of receiving the cap in a first cell of a cuvette during step (b), and creating relative movement between the column and the cuvette so that a second cell of the cuvette collects eluate during chromatography.

13. The method of claim 12, further comprising the step of sensing at least one property of the eluate in the second cell.

14. The method of claim 13, further comprising agitating the eluate in the second cell before the sensing step is conducted.

15. The method of claim 11, wherein the column has longitudinal axis that is vertically disposed when the column is mounted in the support, wherein the decapper member has a plurality of segments, at least one of which slopes with respect to the longitudinal axis of the column, and wherein the decapper member is deflected during step (b).

16. The method of claim 15 wherein the decapper member is a spring element which is flexed during engagement with the tip portion.

17. The method of claim 15, wherein the decapper member is a rigid element which is biassed by a spring which is flexed during engagement with the tip portion.

18. A decapper member for use in the method of claim 15.

19. A method for conducting column chromatography, which comprises the step of utilizing the chromatographic column of claim 1, and further comprising the steps of:

(a) mounting the chromatographic column in a support, with the tip portion being directed generally downward;

(b) introducing a first fluid into the column and collecting a first eluate in a first receptacle that is disposed below the tip portion;

(c) when step (b) is substantially complete, moving the support so as to jar loose any remaining drop of the first eluate;

(d) moving the column above a second receptacle; and

(e) introducing a second fluid into the column and collecting a second eluate in the second receptacle.

20. The method of claim 19, wherein step (c) is conducted by raising the support by less than an inch and then dropping it.

21. The method of claim 19, wherein the support includes a horizontal plate, and wherein step (c) includes lifting said plate.

22. The method of claim 19, wherein the support includes at least one rod, and wherein step (c) includes twisting at least one rod for lifting said plate.

23. The method of claim 19, further comprising the step of moving at least one receptacle to a measuring station, and measuring at least one property of one eluate at the measuring station.

24. The method of claim 23, further comprising the step of simultaneously mixing the first and second eluates with paddles before the measuring step is conducted.

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